CYTOGENETICS AND MOLECULAR ASPECTS OF NONHODGKIN'S LYMPHOMA

BY

SHIBANI BAL

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Dedicated to my mother and father,
Krishna and Makhan.

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ABSTRACT

CYTOGENETICS AND MOLECULAR ASPECTS OF NONHODGKIN'S LYMPHOMA

For the past 10 years there has been a rapid accumulation of data concerning chromosomal abnormalities in lymphoma. In this study, several B-cell types of nonHodgkin's lymphoma have been studied. These include four cases of diffuse, large noncleaved cell lymphoma, three cases of follicular small cleaved cell lymphoma and one case of diffuse large cell immunoblastic lymphoma, diffuse small lymphocytic lymphoma and a case of follicular large cleaved cell lymphoma.

Cytogenetic studies have revealed multiple chromosomal abnormalities in each case. The common t(14;18)(q32;q21) seen in most cases of follicular lympohomas was also observed in all three cases of follicular small cleaved cell lymphoma. The t(14;18) chromosome translocation has also been detected in diffuse large cell, immunoblastic lymphoma and in one case of diffuse, large noncleaved cell lymphoma. Such a finding seems to indicate that these two types of lymphoma (immunoblastic and large noncleaved cell) perhaps started out as a follicular lymphoma but with further changes in their genetic material, they became more of the aggressive type.

The involvement of chromosome 14 is highly significant since the region, 14q32, the site of the immunoglobulin heavy chain locus, is always involved in rearrangements with other chromosomal regions. With the aid of <u>in situ</u> hybridization technique, it was possible

to determine whether chromosome 18 was actually the donor chromosome and chromosome 14 the receiver. By using the *bcl-2* proto-oncogene (located on band 18q21) as a probe labelled with tritium, it was observed that the *bcl-2* gene had moved to band 14q32. Both the follicular lymphoma and the immunoblastic lymphoma exhibited a higher accumulation of grains on band 14q32. Southern blot analysis also showed the *bcl-2* gene rearrangement in the case of diffuse, large noncleaved cell lymphoma. Therefore, it is possible that the juxtaposition of the *bcl-2* gene to the immunoglobulin heavy chain locus may lead to neoplastic development of these types of B-cell lymphomas.

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LIST OF ABBREVIATIONS

BL	Burkitt's lymphoma.
IGH	Immunoglobulin heavy chain gene.
NHL	NonHodgkin's lymphoma.
PBL	Peripheral blood lymphocytes.
λ	lambda.
κ	kappa.

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I. INTRODUCTION

In the past decade the study of leukemia has leaped forward with tremendous strides, especially in the study of chronic myelogenous leukemia(CML). Cytogenetic study of other cancers, such as solid tumors has lagged somewhat behind. This is due to the fact that chromosomal studies in leukemia are much easier since cells are fairly easy to culture. Whereas for solid tumors, primary cultures are more difficult to set up. Primary cultures of tissue biopsies are sensitive to the treatment for culturing. Because good metaphase spreads are required for cytogenetic analysis, the study of solid tumors has not progressed as far as that of leukemia (Sandberg, 1981).

The importance of studying solid tumors cytogenetically is that it will allow us to see the types of genetic changes that occur within the chromosomes as the cell begins to behave abnormally. Though histological studies can show the morphological changes, the definitive chromosomal changes would have already occurred and the primary chromosomal changes would not be detected. Cytogenetic analysis will enable us to determine the primary changes that occur within the chromosomes.

Malignant lymphoma is a neoplastic proliferative process of the lymphoreticular portion of reticuloendothelial system involving cells of either the lymphocytic or histiocytic series in varying degrees of differentiation. The lymph nodes, the spleen, the thymus, the gut-associated lymphoid tissues, the bone marrow in its nonhemopoietic function and scattered

macrophages and lymphocytes elsewhere make up the lymphoreticular tissues (Lukes, 1968).

Studies in cancer research, especially in the cytogenetic and molecular aspects, have revealed many chromosomal anomalies. Majority of the work has been done on leukemia, and the first conclusive evidence relating chromosomal aberration with a particular cancer was demonstrated by Nowell and Hungerford (1960) in CML patients.

The first chromosomal anomaly reported in lymphomas involved chromosome number 14. This was reported by Manolov and Manolova in 1972 in Burkitt's lymphoma (BL). With the aid of banding techniques a translocation involving chromosome 8 and 14 was detected (Manolova et al., 1979). Other less common translocations found in variant Burkitt's lymphoma occur as t(8;22)(q24;q11) and t(2;8)(p11;q24) (Van Den Berghe et al., 1979, Berger, et al., 1979).

With the advent of molecular genetic studies, using techniques for DNA analysis and the discovery of cellular oncogenes, it became possible to describe the probable nature of these translocation. About 60 cellular oncogenes have now been identified largely through the auspices of rapidly transforming retroviruses(Burck et al., 1988).

Through the use of in situ hybridization technique it has been shown specifically in BL that the cellular oncogene, *c-myc*, located in region 8q24 is somehow involved in cellular transformation (Dalla-Favera et al., 1982). This has been verified through immunological studies of the expression of light chains of the immunological type. It has been found that gene sequences coding for heavy chains of immunoglobulins are

localized on 14q32 (Croce et al., 1979). Genes coding for kappa (κ) light chains are on chromosome 2p11 (McBride et al., 1982; Malcolm et al., 1982). Lambda (λ) light chains are localized on chromosome 22q11 (Erikson et al., 1981).

In the variant translocations of BL, λ chains are expressed in cells with t(8;22). Kappa chains are expressed in cells with t(2;8), and either λ or κ is expressed in t(8;14). This was first pointed out by Lenoir et al., 1982.

Again the use of in situ hybridization has allowed the visualization that *c-myc* remains on chromosome 8 in t(2;8) and t(8;22), but in t(8;14), *c-myc* region is translocated to chromosome 14 (Hamuster, 1986). In most cases, the *c-myc* gene, has been found rearanged head-to-head (5'-5') with Cμ, but variations do exist (Taub et al., 1982; Dalla-Favera et al., 1983).

Similar studies on other types of lymphoma have shown various abnormalities. The involvement of *c-onc* is increasingly being reported and in many translocations it appears that these *c-onc* genes are being translocated. The most significant discovery made in these studies, showed that chromosome 14 is always involved in these translocations, specifically at band 14q32. This seems to indicate that the translocation occuring at the IgH region may play a major role in cellular transfomation.

It has also been observed that there is a striking correlation between a particular translocation and the histologic subtypes of malignant lymphoma. The t(14;18) chomosomal translocation has been consistantly observed in follicular lymphoma, specifically in follicular small cleaved cell lymphoma. Many more consistent chromosomal abnormalities are being cited in other types of lymphoma.

In this study, the cytogenetic and molecular aspects of nonHodgkin's lymphoma have been examined. The cytogenetic results have been correlated with their histopathology. Molecular studies have been done to describe oncogene movement, specifically *bcl-2* gene movement.

Oncogene movement is being studied since the movement of such genes have been demonstrated to be involved in some way to cause cells to behave abnormally. In this study, only the *bcl-2* gene located on chromosome 18q21 has been used. This gene has been observed to be involved in many types of nonHodgkin's lymphoma.

The correlation of cytogenetics and the histopathology will enable the diagnosis of 'hard to diagnose' cases of nonHodgkin's lymphoma. By studying the chromosomal changes occuring and by examining the histology it will be possible to describe the progress of the disease. In other words, it will be possible to see what cytogenetic changes are associated with specific morphological changes that are being observed.

II. LITERATURE REVIEW

II. 1. Normal B-cell Development

In order to understand the neoplastic process of nonHodgkin's lymphoma which primarily involves B-cells, though T-cells may be involved, it is necessary to study the normal developmental and differentiating processes of the B-cells. When pre-B-cells differentiate into immunoblasts, which then form immunoglobulin producing plasma cells there are many possibilities that something may go wrong and differentiation may stop, and increased

mitosis will occur.

B-cell development begins initially as does other lympho-reticular cells, in the bone marrow. These cells are mostly progenitor of stem cells which will give rise to the different types of cells in the lympho-reticular system.

There are three proliferative stages in the natural history of B-lymphocytes (Melchers and Potter, 1987). The first proliferative stage involves cell division that is associated with the differentiation of membrane bound immunoglobulin pre-B cells from stem cells. The second proliferative stage involves cell division that results from activation of pre-B cell to immunoglobulin secreting cells. In the final proliferative stage of B-cell development, cell division occurs from the secondary activation of resting, G_o, cells that have derived from the second stage of immunoglobulin-secreting cells.

Cells that derive from the first and second proliferative stages can either enter a mitotically inactive G_o phase or they can go into the subsequent proliferative stage. Proliferative second and third stage cells will then become the immunoglobulin secreting cells. A majority of these cells are eliminated within a few days. Those that remain can live much longer. This change to longevity may be induced by antigenic selection at specific sites (Melchers and Potter, 1987).

In stage one of the proliferative stages, immunoglobulin heavy and light chain genes rearrange in orderly steps to form the template for the light and heavy chain synthesis. This establishes the initial clonal characteristics of the B-cell.

These cells carrying their specific immunoglobulin, as a membrane receptor, enter the circulation. Those

cells that do not carry functional immunoglobulin are eliminated. Newly formed B-cells are continuously entering and leaving the circulation.

When these cells, which are at G_o, encounter an antigen, or other appropriate exogenous signals, they begin to proliferate. During the second stage of proliferation, the cells undergo immunoglobulin heavy chain switching. It is possible for somatic mutations to occur at this stage of proliferation. Those cells that are not eliminated, can enter G_o or go directly into G_1, the third proliferative stage of development. Cells that escape the influence of the antigenic agents enter a mitotically inactive, G_o, stage. The switched or mutated cells become the progenitors for the third stage of proliferation (Melchers and Potter, 1987).

The proliferative stages of B-lymphocyte differentiation are important to B-cell neoplastic development because active cell proliferation, i.e., mitotic division and the passage of cells through G_1, S, G_2 stages and mitosis, is usually required for the fixation of mutations, the integration of retroviruses and the formation of chromosomal translocations. It is during the proliferative stages that mutagenic events are first expressed and proliferation may also be required for the selection of mutant cell phenotypes.

The type of nonHodgkin's lymphoma that occurs depend on where B-cell differentiation and maturation is arrested. Melchers and Potter (1987) describe this as the maturational arrest phenomenon.

They describe it in two ways. It could be due to a failure in the activation of a gene whose product is required to activate another gene or genes that must be operating in the next stages. Also maturation may be

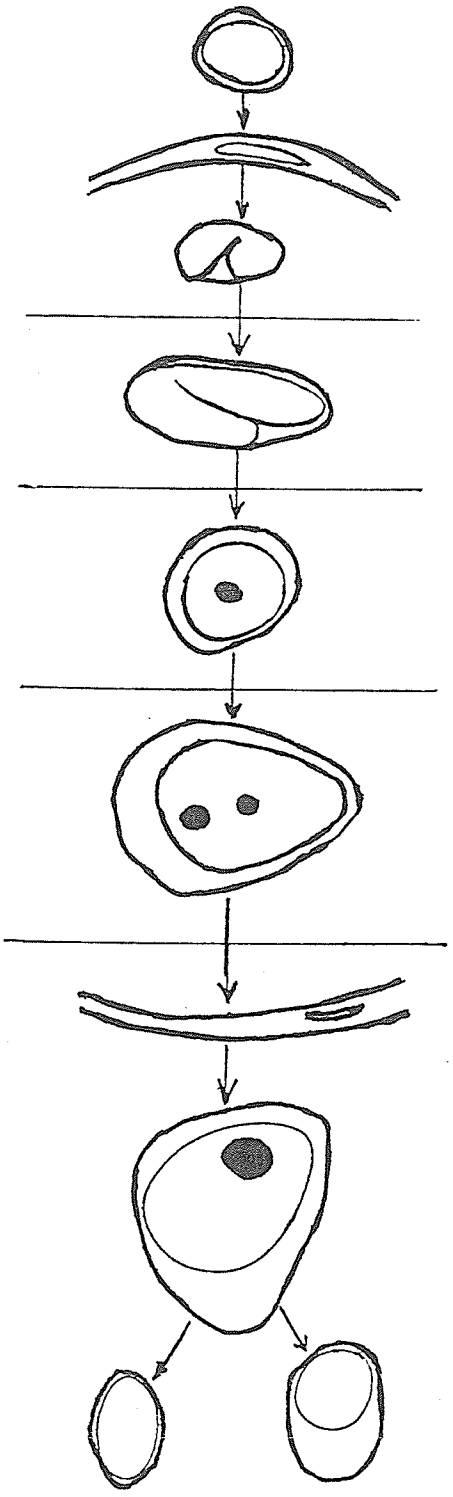
blocked and cells cannot enter the G_o stage, and therefore continue to proliferate. Normally, B-cell maturation occurs in the follicles of the lymph nodes.

There are four morphological stages of B-cell development (Figure 1). These are the small cleaved cell stage, the large cleaved cell, the small noncleaved cell and the large noncleaved cell stage (Robbins et al. 1984). The first cell stage is the small cleaved cell stage. At this stage the cell contains very little cytoplasm. The nucleus is convoluted and appears cleaved in tissue sections. The second stage is the large cleaved cell stage. More cytoplasm is apparent at this stage. Also, nucleoli begin to appear. Mitotic activity is low during the first and second stage. The third stage is the small noncleaved cell stage. The nucleus becomes spherical and mitotic activity increases. The fourth differentiation stage is the large noncleaved cell stage. The nucleus is round and more cytoplasm is visible. From here, cells enter the interfollicular space and become immunoglobulin producing cells.

Both the proliferative stages and the morphological stages of B-cell development have to be examined together, since they are describing the same events. The morphology of B-cell development have to be studied, since it is the morphological terms that is being used to clinically describe the different types of B-cell lymphomas.

Figure 1. Schematic representation of the four morphological stages of B-cell development.

Follicular Center Cell Transformation



"B" Lymphocyte

Small Cleaved Cell

Large Cleaved Cell

Small Noncleaved Cell

Large Noncleaved Cell

Immunoblast

Plasma Cells

Interfollicular Area

II. 2. NonHodgkin's Lymphoma

Lymphoma has a striking correlation with leukemias since both involve lymphocytes. Leukemia is also a lymphoreticular disease. Traditionally, the term leukemia has been applied to all processes with numerous abnormal or neoplastic cells in the peripheral blood. This widely accepted definition is based upon diffuse involvement of the bone marrow by specific type of neoplastic cellular proliferation which is associated with uniform involvement of spleen, liver and lymph nodes. Where as the distribution of lymphomas is irregular and variable, and may not be involved or exhibit irregular nodular involvement. Lymphomas, with progression of the disease, tend to become more widespread and approach the leukemic distribution (Lukes, 1968).

Lymphomas occur essentially in a homogeneous population of a single cell type; when mixtures are found, they appear to represent variations in the size or configuration of a single cell type (Lukes, 1968). The character of histologic involvement is either diffuse (uniform) or follicular (nodular), and the distribution of involvement may be irregular or systemic (generalized) (Figures 2a and 2b).

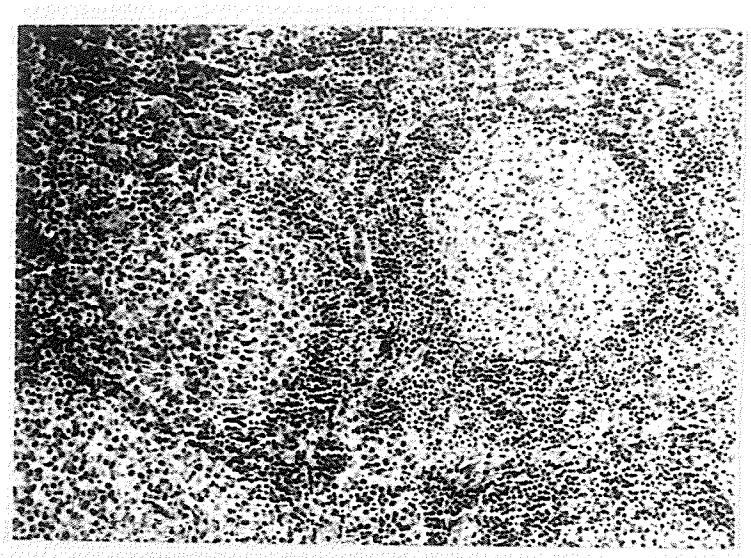
Using the four morphological stages of B-cell development, a Working Formulation for clinical use has been developed (Working Formulation, 1982). In 1982, an international panel of experts suggested a new classification that would assemble the four morphological catagories of NHL into three prognostic groups .

The international Working Formulation is based

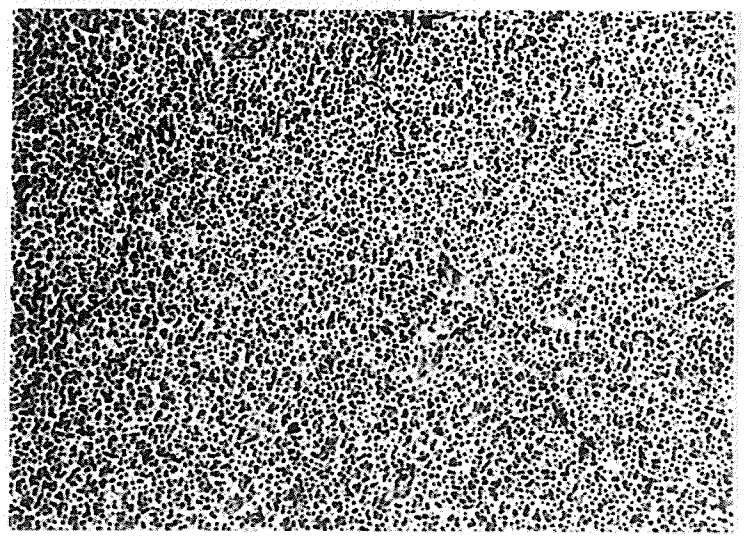
Figure 2a: Tissue section showing follicular pattern.

Figure 2b: Tissue section showing diffuse pattern.

a



b



purely on the morphology of the cells. The three prognostic groups being the low, intermediate and high grade nonHodgkin's lymphoma. All other forms that cannot be placed according to the four morphological catagories are placed under miscellaneous.

To gain a better understanding of the malignancy process, since it does involve the genetic expression of these cells, it is obvious that the study of the nuclear material at the chromosomal and DNA level is important. This research will eventually lead to the illucidation of the mechanisms involved in the malignancy process.

II. 2.1. Types of NonHodgkin's Lymphoma

As described previously the types of NHL is classified according to the Working Formulation (WF). NonHodgkin's lymphomas are placed under four catagories. This is the *low grade*, the *intermediate grade*, the *high grade*, and *miscellaneous*.

The low grade include the histological subtypes such as small lymphocytic, follicular small cleaved, follicular mixed and follicular large cell lymphomas (Mead, 1990). These lymphomas disseminate early. The involvement of widespread nodal site, the liver, spleen and bone marrow are common.

The intermediate grade lymphomas include such histological types as follicular large cell, diffuse small cleaved, diffuse mixed, and diffuse large cell lymphomas.

The high grade lymphomas include the large cell, immunoblastic, the lymphoblastic and the small noncleaved cell lymphomas.

The miscellaneous group of lymphomas are other types

of lymphomas that cannot be placed under the three catagories. The characteristics that define the type of lymphoma do not always fit the critaria required to be placed in the three catagories defined by the Working Formulation (Table 1).

Table 1

A Working Formulation of Non-Hodgkin's Lymphomas

Working Formulation

LOW GRADE

A. Malignant lymphoma
   Small lymphocytic
   consistent with CLL
   plasmacytoid

B. Malignant Lymphoma, follicular
   Predominantly small cleaved cell
   diffuse areas
   sclerosis

C. Malignant Lymphoma, follicular
   Mixed, small cleaved and large cell
   diffuse areas
   sclerosis

INTERMEDIATE GRADE

D. Malignant Lymphoma, follicular
   Predominantly large cell
   diffuse areas
   sclerosis

E. Malignant lymphoma, diffuse
   Small cleaved cell
   sclerosis

F. Malignant lymphoma, diffuse
   Mixed, small and large cell
   sclerosis
   epithelioid cell component

G. Malignant lymphoma, diffuse
   Large cell
   cleaved and noncleaved cell
   sclerosis

Table 1-continued

HIGH GRADE

H. Malignant lymphoma
Large cell, immunoblastic
plasmacytoid
clear cell
polymorphous
epithelioid cell component

I. Malignant lymphoma
Lymphoblastic
convoluted and nonconvoluted

J. Malignant lymphoma
Small noncleaved cell
Burkitt's
follicular areas

MISCELLANEOUS

Composite
Mycosis fungoides
Histiocytic
Extramedullary plasmacytoma
Unclassifiable
Other

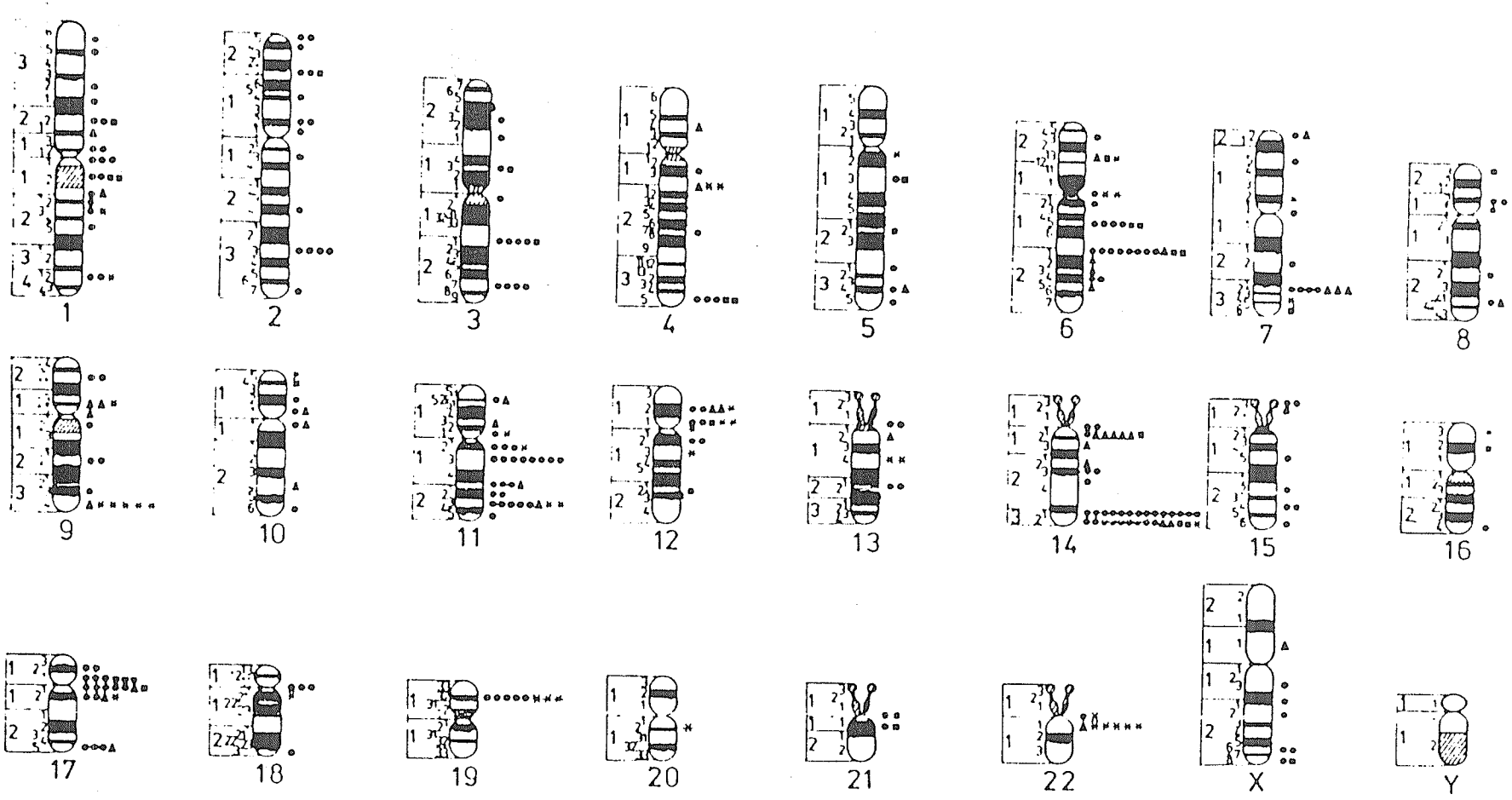
II. 3. Cytogenetics of NonHodgkin's Lymphoma

Since Manolov and Manolova's discovery of the 14q+ marker chromosome in Burkitt's lymphoma, cytogenetic data has been accumulating rapidly. This is due to improvements in culturing and banding techniques. As data acculmulates, a pattern is beginning to appear in which certain, specific abnormalities are being detected in different types of NonHodgkin's Lymphoma (NHL). For example, as discussed previously in Burkitt's lymphoma, the appearance of t(8;14)(q24;q42) is constant, as well as the t(2;8) and the t(8;22) chromosomal translocations in the variant forms of Burkitt's lymphoma (Abe et al.,1982; Berger et al., 1983).

Non-Hodgkin's lymphoma exhibits a heterogeneous population of cells. More than one clonal population of cells have been observed cytogenetically. All 22 pairs of autosomes have been observed to be involved. The sex chromosomes are sometimes involved as well (Figure 3). Both structural and numerical abnormalities have been observed in all cases of NHL (Berger et al., 1984; Donti et al., 1987; Fleishman et al., 1989; Fraisse et al., 1984; Gaunt et al., 1986; Kaneko et al.,1982; LeBeau et al.,1984; Levine et al., 1985; Levine, et al.,1989; Mark et al.,1978; Ohyashiki et al., 1985; Panami et al., 1984; Reeves et al., 1989; Speaks et al., 1987; Tilly et al., 1988; Yunis et al.,1982). Chromosomes most often involved in NHL are chromosomes 1, 6, 11, 12, 14, and 18 (Table 2).

In NHL, studies indicate that there is perhaps one primary chromosomal aberration and subsequent abnormalities are secondary. As the pre-B-cell goes through the stages of development, some external pressure may cause abnormal rearrangements to occur, causing the

Figure 3: Chromosome map showing breakpoint clusters seen in ML and ALL.



Clustering of breakpoints in rearranged chromosomes in ML and ALL: ● ML; ■ ML with marrow blastosis; * ALL; ▲ ALL with extramedullary tumor nodes. Some of these cases could be ML with early generalization

Table 2

CHROMOSOMES MOST OFTEN INVOLVED IN NHL

Chromosome Number	Abnomalities
1	partial duplication of q dup(1)(q) with t(8;14) deletion or translocation
6	20.5% involves the q arm del(6)(q) breaks at 6q15 and 6q27 loss of q arm with dup(6)(p)
11	t(11;14)(q13;q32) Numerical and structural involvement of 11 other than the t(11;14)
12	structural abnormalities rather infrequent trisomy 12 in most cases associated with several other changes
14	t(8;14), t(11;14), t(14;18)
18	t(14;18) is the major trisomy 18 del(18)(q) breakpoint at 18q21 and 18q23

cell to stop differentiation and to proliferate at that particular stage.

As discussed earlier, the chromosomal translocation t(8;14), was the first observed consistent abnormality for a particular type of NHL. The second such consistent abnormality observed in a B- cell type NHL is the t(14;18)(q32;q21) chromosomal translocation found in follicular lymphomas (Tsujimoto et al., 1984).

In 85% of the cases of follicular small cleaved cell lymphoma, the presence of t(14;18) has been observed (Saltman et al., 1988). This perhaps is an indication that the t(14;18) chromosomal translocation may be a primary event occurring at the very early stages of B-cell maturation. As other chromosomal rearrangements occur, the lymphoma may become more aggressive and become diffuse and metastasize (Armitage et al.,1988).

II. 4. Molecular Aspects of Non-Hodgkin's Lymphoma

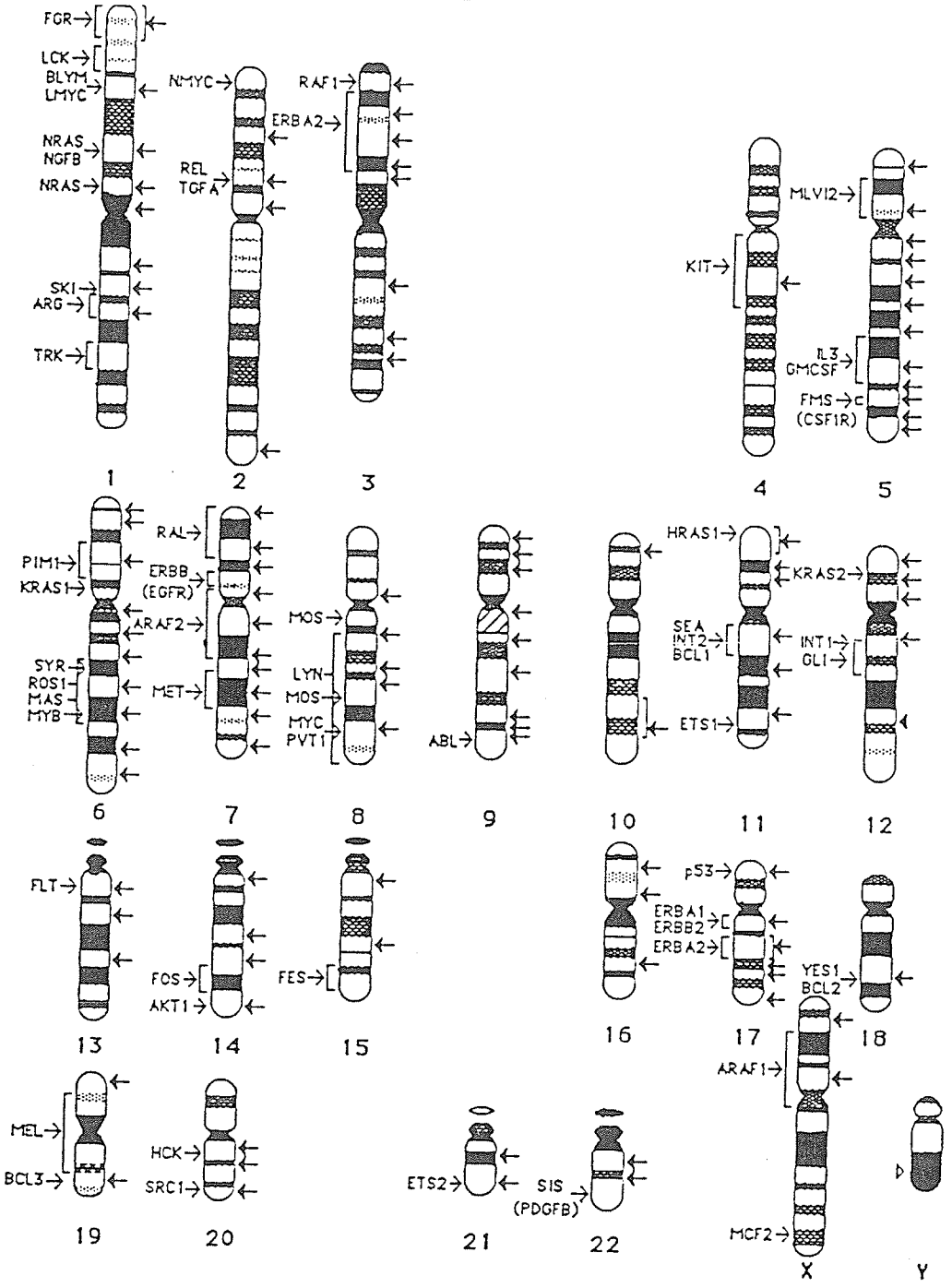
From the cytogenetic amd molecular studies, it is now apparent that the sites of consistent translocations pin-point chromosomal segments that contain genes critical in malignant transformation. With advent of molecular genetic studies using techniques for DNA analysis and the discovery of cellular oncogenes (c-onc), it is possible to describe the possible nature of these translocations (Griesser et al.,1989).

I. 4.1. Oncogenes

There is increasing evidence to implicate oncogenes in the etiology of NHL. Some of these oncogenes have been identified because of their location at common breakpoints, some because they have been found by transfection assays to be activated, and some because

Figure. 4: Map of chromosome location of proto-oncogenes (Rowley, 1990).

Fig. 4


FGR
LCK
BLYM
LMYC
NRAS
NGFB
NRAS
SKI
ARG
TRK
1
NMYC
REL
TGFA
ERBA2
2
RAF1
3
KIT
4
MLVI2
IL3
GMCSF
FMS
(CSF1R)
5
PIM1
KRAS1
SYR
ROS1
MAS
MYB
6
RAL
ERBB
(EGFR)
ARAF2
MET
7
MOS
LYN
MOS
MYC
PVT1
8
ABL
9
HRAS1
SEA
INT2
BCL1
ETS1
10
11
KRAS2
INT1
GLI
12
FLT
13
FOS
AKT1
14
FES
15
p53
ERBA1
ERBB2
ERBA2
16
17
YES1
BCL2
18
MEL
BCL3
19
HCK
SRC1
20
ETS2
21
SIS
(PDGFB)
22
ARAF1
MCF2
X
Y

they have been shown to be expressed in NHL. So far there is no consistent pattern of involvement between specific oncogenes and specific subtypes of NHL. This may be because more than one oncogene is necessary for transformation (Chenevix-Trench, 1987).

Over 60 proto-oncogenes or genes with transforming properties have now been mapped (Figure 4). These genes are known to acquire their transforming properties either through amplification of the particular gene, deletion, translocation of the gene to other sites, point mutations, or by viral promoter inserting next to the gene (Alitalo et al., 1986; Cory, 1986; Nowell, 1990).

Analysis of the DNA sequences at the chromosome breakpoints of several of the recurring translocations in leukemias and lymphomas has resulted in identification of the genes adjacent to the breakpoints. The t(8;14) t(11;14) (Tsujimoto, et al., 1985a) and t(14;18) are the three most frequently occurring translocations in NHL (Figure 5). These translocations result in deregulation of transcription of the affected genes. In addition, the genes appear to have normal functions that, when perturbed, might be expected to contribute to the development of neoplasia. Other proto-oncogenes are known to be involved in NHL such as the *bcl-1*, *c-ets-1* and *c-ets-2*, *c-dbl*, *c-Tlym-1*, *c-N -ras* and *c-Blym-1* (Chenevix-Trench, 1987).

II. 4. 1.1 The *c-myc* gene

The *c-myc* gene located on chromosome band 8q24 and the *bcl-2* gene located on chromosome band 18q21 have been analyzed in full detail. It has been demonstrated that the translocations result in aberrant regulation of the

Figure. 5: Translocation involving chromosomes 8, 11, and 18 with chromosome 14. Small arrows indicate the oncogene site and the IgH site.

Fig. 5

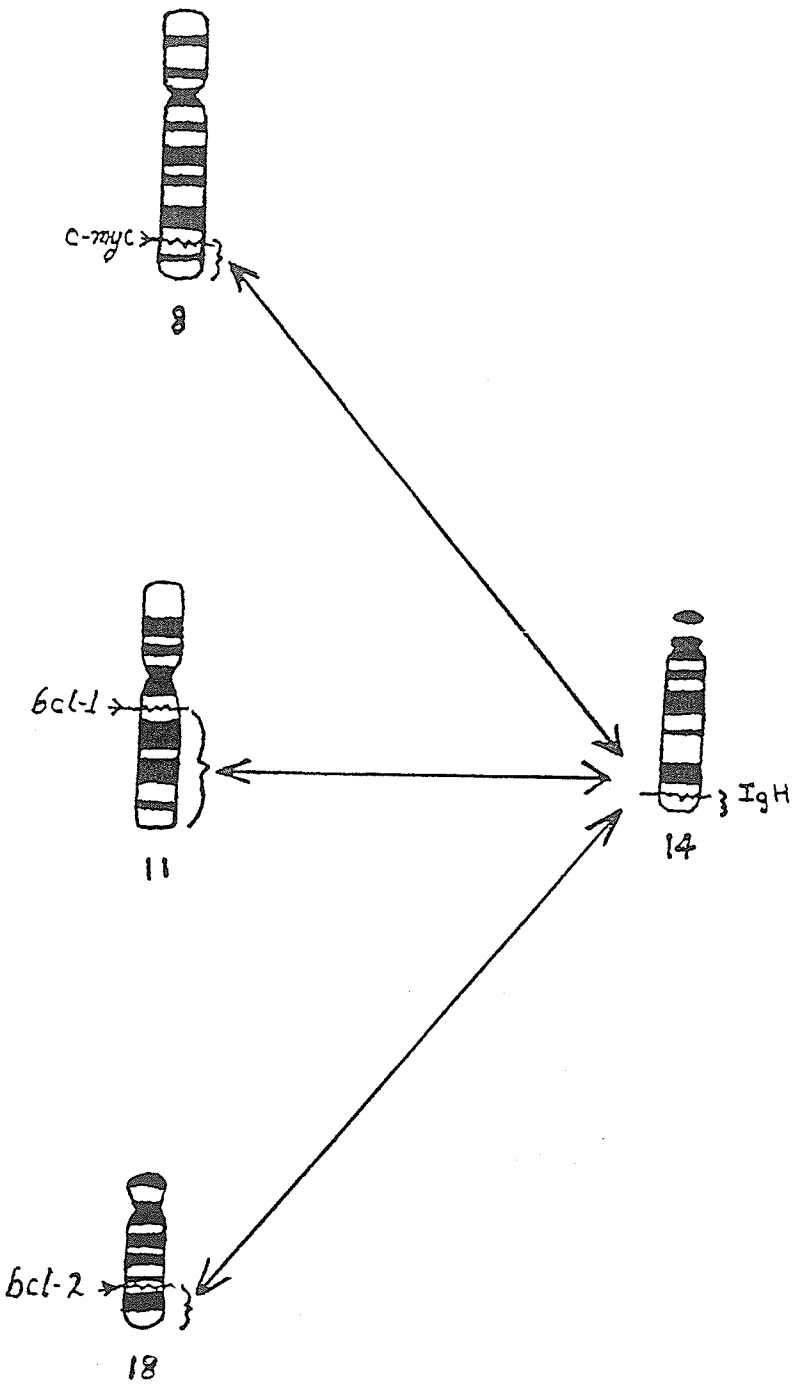
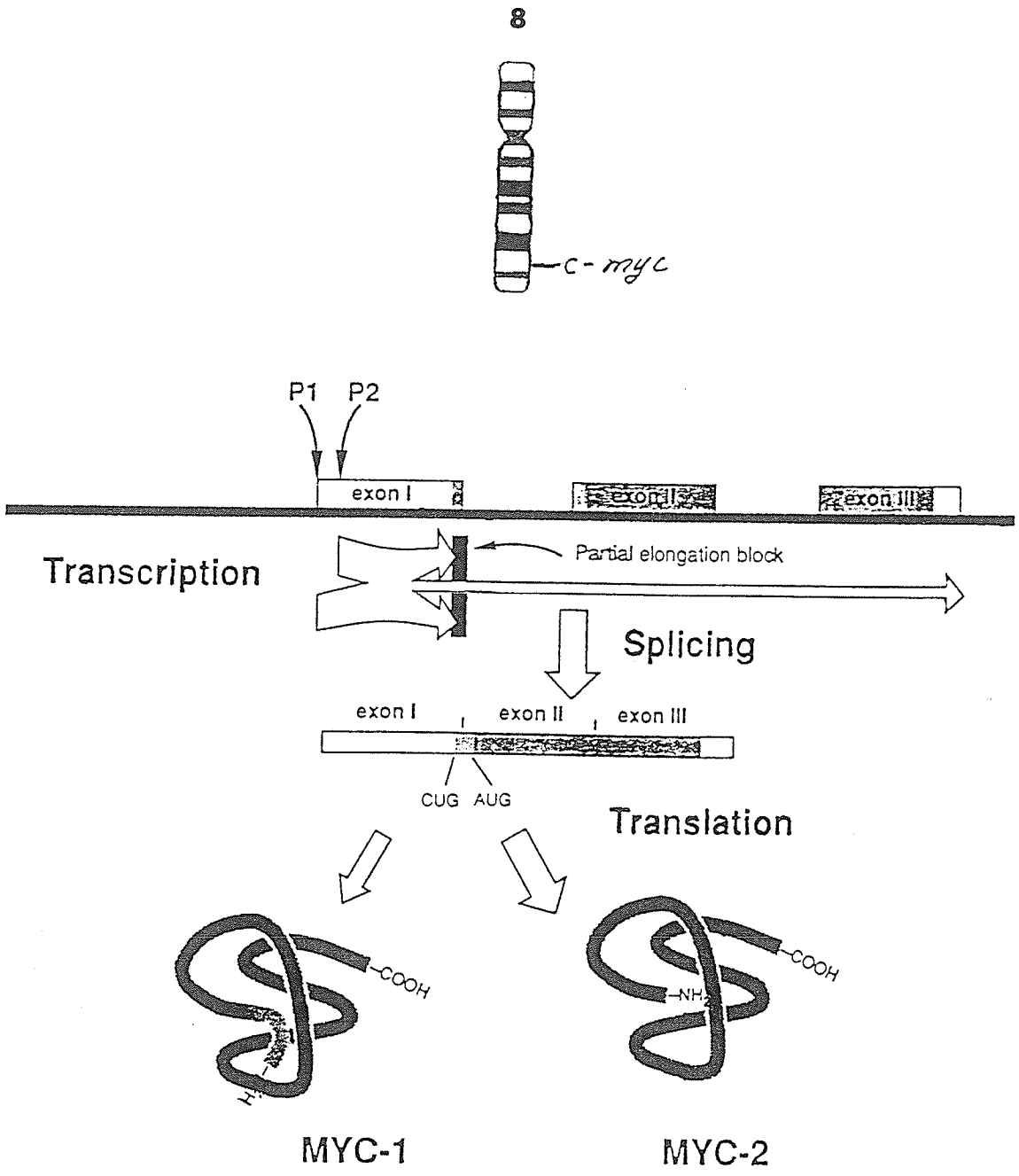


Figure 6: *c-myc* gene

The structure of the *myc* gene and the steps involved in synthesis of the protein products. At the top are shown the three *myc* exons. The transcriptional start sites of the two major promoters are illustrated with arrows (P1 and P2). In the normal *myc* gene, the majority of transcripts terminates early, before the regions are transcribed. After splicing, the mRNA is transported to the cytoplasm, where its coding information specifies the synthesis of two proteins (myc-1 and myc-2). The major product, myc-2, is formed if translation begins at an AUG codon near the beginning of the second exon; initiation from a CUG codon near the end of the first exon results in a slightly larger protein with identical sequence except for the addition of 14 or 15 amino acids at the amino terminus (McKeithan, 1990).


8
c-myc
P1
P2
exon I
exon II
exon III
Transcription
Partial elongation block
Splicing
exon I
exon II
exon III
CUG
AUG
Translation
COOH
NH2
COOH
MYC-1
MYC-2

synthesis of the protein product of the *myc* and the *bcl-2* genes, respectively.

The *c-myc* gene is made up of 3 exons and only the second and third exons encode the *myc* polypeptide which has DNA binding properties. Transcription is initiated at two sites on exon I, resulting in two mRNA's. The two *c-myc* phosphoproteins have a molecular mass of 64 kDa and 67kDa (Figure 6).

Studies of *myc* expression in BL cells show that a decrease in protein synthesis will increase *myc* mRNA levels, indicating that *myc* expression is negatively regulated. This protein could act either by repressing transcription or by acceleration of *myc* mRNA degradation. Dani et al. (1984) have shown that *myc* mRNA is very unstable; having a half-life of 15 minutes in both normal and transformed cells. This indicates that *myc* expression is controlled through the degradation of its message, but it is still possible that transcriptional control exists as well. The nuclear location, DNA binding capacity and a short half-life of *c-myc* protein (30 minutes) suggest that it is involved in control of gene expression (Cory, 1986).

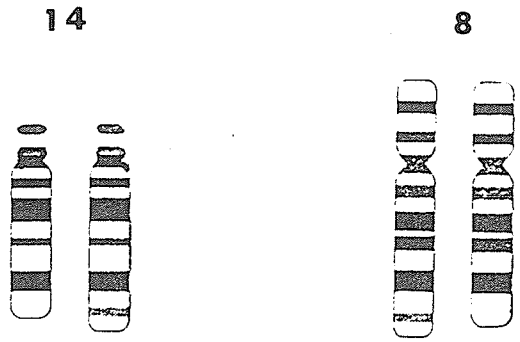
Molecular studies have shown that chromosome 14 with the more distal variable region genes are being moved to chromosmome number 8. In most cases, the *c-myc* gene has been found rearranged head to head (5'-5') with the $C\mu$ but variations do exist. The only consistency is that the *c-myc* gene always ends up upstream of an Ig constant region. The *c-myc* breakpoint may be scattered on either side of the first exon, often being far from the structural gene. The breaks on chromosome number 14 generally are in the μ-switch region (Figure 7).

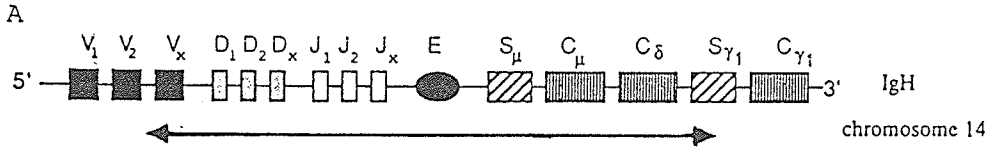
Figure 7: Translocation of *c-myc* to IgH.

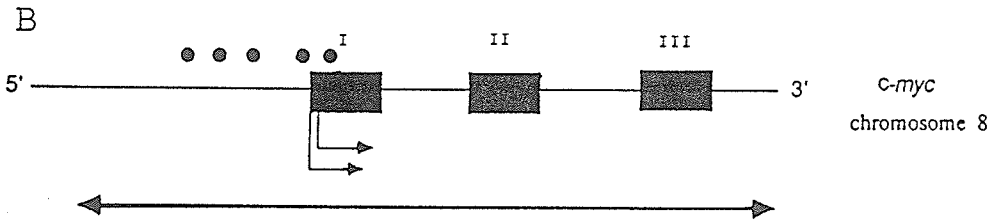
A Representation of the IgH locus (A), the *c-myc* locus (B), and an example of t(8;14) BL translocation (C). The V, D, J, regions are portions of the variable region gene; E is the enhancer region, S is the switch region and C regions are the constant region genes.

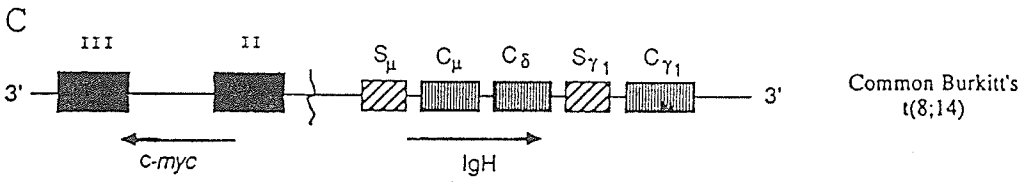
B The exons of *c-myc* are labeled I, II, and III. The direction of transcription from the two promoters in exon 1 is shown by the arrowed lines. The black dots represent DNase I hypersensitive sites. The double headed arrows on A and B represent the region in which breaks occur in BL.

C The IgH chain gene and the *c-myc* oncogene in an example of t(8;14) in BL. The *myc* exons, II and III, are marked, as are the switch and constant regions of the IgH gene. The arrows indicate the direction of transcription.


14
8


A
V1 V2 Vx D1 D2 Dx J1 J2 Jx E Sμ Cμ Cδ Sγ1 Cγ1
5'
3'
IgH
chromosome 14


B
I
II
III
5'
3'
c-myc
chromosome 8


C
III
II
Sμ Cμ Cδ Sγ1 Cγ1
3'
3'
c-myc
IgH
Common Burkitt's
t(8;14)

II 4. 1.2 The *bcl-2* gene (Figure 8)

The *bcl-2* gene undergoes chromosomal translocation in 85% of cases of follicular lymphomas, 20% in diffuse large cell lymphoma and 10% in CLL of B-cells (Cleary et al., 1985; Aisenberg et al., 1988; Seto et al., 1988; Lee et al., 1987).

Translocation of *bcl-2* gene from chromosome 18 to the J_H segment of the Ig gene at chromosome band 14q32 in B cells results in deregulated expression of this gene, causing high steady state levels of *bcl-2* mRNA (Figure 9). This gene rearrangement is due to mistakes in VDJ joining (Tsujimoto, et al., 1985b). This results in the juxtapositioning of *bcl-2* gene and brings it under the control of the IgH. Under normal conditions, the level of *bcl-2* mRNA is high during pre-B-cell development. But as the cell matures or approaches the resting cell stage the *bcl-2* mRNA level is down regulated (Graninger et al., 1987, Chen-Levy et al., 1989, McDonnell et al., 1989, Reed et al., 1989). As the cell matures its immunoglobulin mRNA levels will rise. If at this point the *bcl-2* gene is brought within the IgH gene, the *bcl-2* will be transcribed, leading to increased transcript levels. DNA sequence data indicate that *bcl-2* encodes two proteins by virtue of alternative splicing, designated as Bcl-2α and Bcl-2β, which have relative masses of 26kDa and 22kDa, respectively (Reed et al., 1987). Fractionation experiments indicate that the Bcl-2α is located at the inner surface of the cell membrane, suggesting a possible role in mitogenic signal transduction (Halder et al., 1989).

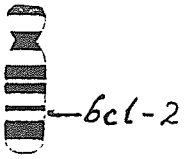
Studies show that breaks can occur on the 5' or 3' region of the *bcl-2* gene. This gene consists of at

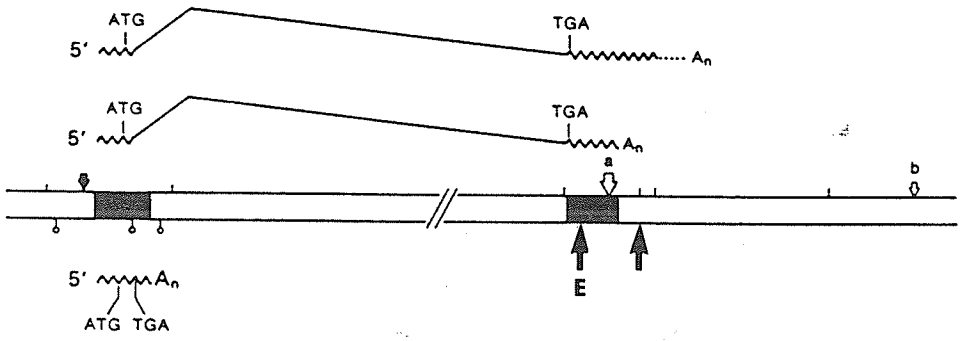
Figure 8: *bcl-2* gene

Genomic organization of the *bcl-2* gene. The filled boxes represents two exons of the *bcl-2* gene. Three different mRNAs, 8.5 kb, 5.5 kb and 3.5 kb, are also shown by wavy lines. The polyadenylation site for 8.5 kb is not precisely mapped, as shown by the dotted line. The two breakpoint hot spots of the t(14;18) translocation are shown by open arrows. The restriction sites are shown by | for Hind III, and ¦ for BamHI (Melchers and Potter, 1987).

Bold arrows indicate genomic 3.5Kb EcoRI-Hind III fragment used as a probe in this study. EcoRI site is designated as E.

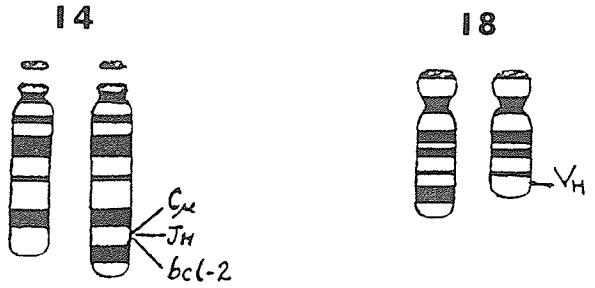
18

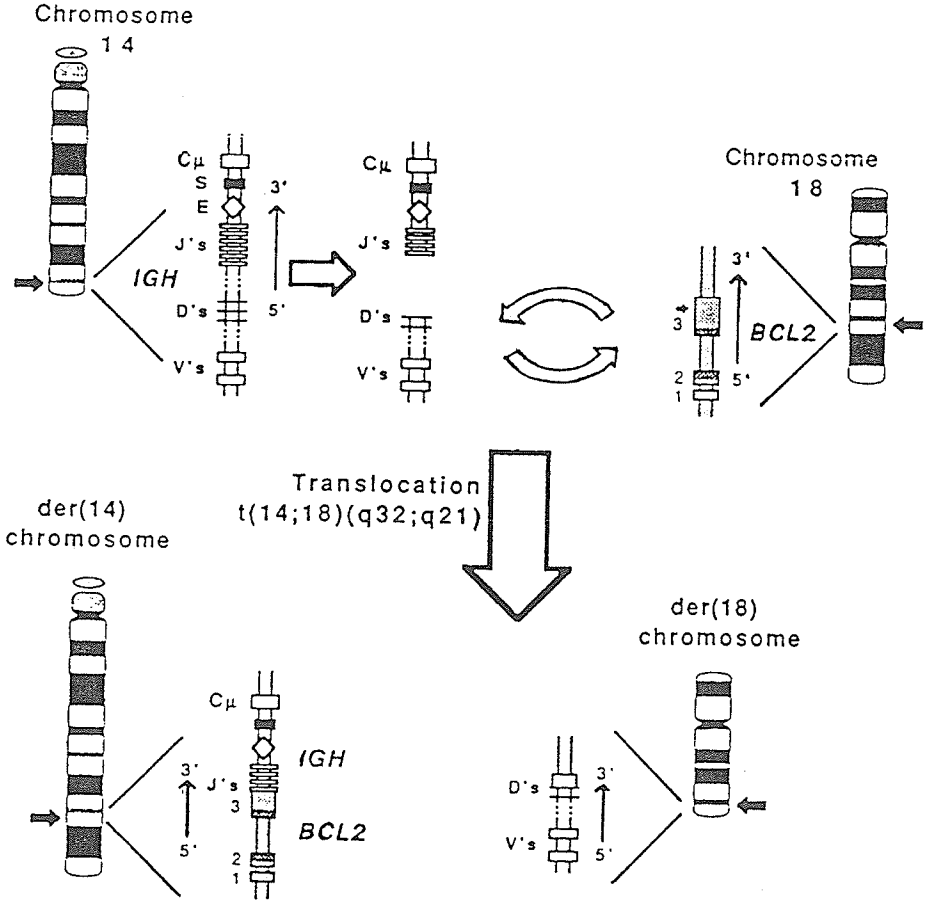




least three exons. The major breakpoint region is clustered in the 3' noncoding region (third exon) of the *bcl-2* gene (McKeithan, 1990). The minor breakpoint region is clustered at a region 3' to the *bcl-2* gene.

Figure 9: Translocation of *bcl-2* gene to IgH
Arrows indicate the breakpoints. The translocation appears to occur during D-J joining of the IgH gene.


14
18
Cμ
JH
bcl-2
VH


Chromosome
14
Cμ
S
E
J's
IGH
D's
V's
3'
5'
Cμ
J's
D's
V's
Chromosome
18
3'
3
2
1
5'
BCL2
Translocation
t(14;18)(q32;q21)
der(14)
chromosome
Cμ
IGH
3'
J's
3
BCL2
5'
2
1
der(18)
chromosome
D's
V's
3'
5'

II. 5. The Immunoglobulin Genes

Through many studies, it seems that the driving force in B-cell neoplasia appears to be the immunoglobulin (Ig) genes (Melchers and Potter,1987) Predominantly the involvement of the immunoglobulin heavy chain (IgH) gene has been observed. For many of the B-cell lymphomas, the Ig gene loci provides the substrate for many mutational events.

As demonstrated in BL the t(2;8) translocation, the kappa (κ) immunoglobulin gene is involved. In the t(8;22) the lambda (λ) immunoglobulin gene is involved. The most common chromosomal translocation which is the t(8;14) involves the immunoglobulin heavy chain (IgH) gene. The κ gene is located on chromosome 2 band p11. The λ-chain gene is located on chromosome 22 band q11. The IgH locus is on chromosome 14 band q32.

It is because of the discovery of the t(8;14) chromosome that the study of the IgH chain gene was initiated. In trying to characterize the specific regions involved in this chromosomal translocation, it was found that the *c-myc* oncogene translocated to chromosome 14 band q32, the site of the IgH locus. Since then (Capra and Tucker, 1989) much more pertinent information has been gathered about the IgH locus, not just the types of genes that make up this region, but also the sequences that make up these regions.

The locus contains at least 200 variable (V_H) genes. There are well over 20 diversity (D) regions. A constant heavy chains (C_H) region, consisting of nine functional genes have been located. These genes consist of the μ, δ, γ_3, γ_1, α_1, γ_2, γ_4, ε, and α_2. This region also contains two pseudogenes $\psi_{\varepsilon 1}$ and ψ_{γ}. Also there are six

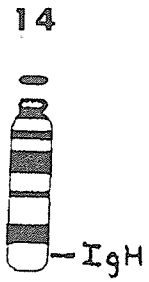
joining (J_H) segments.

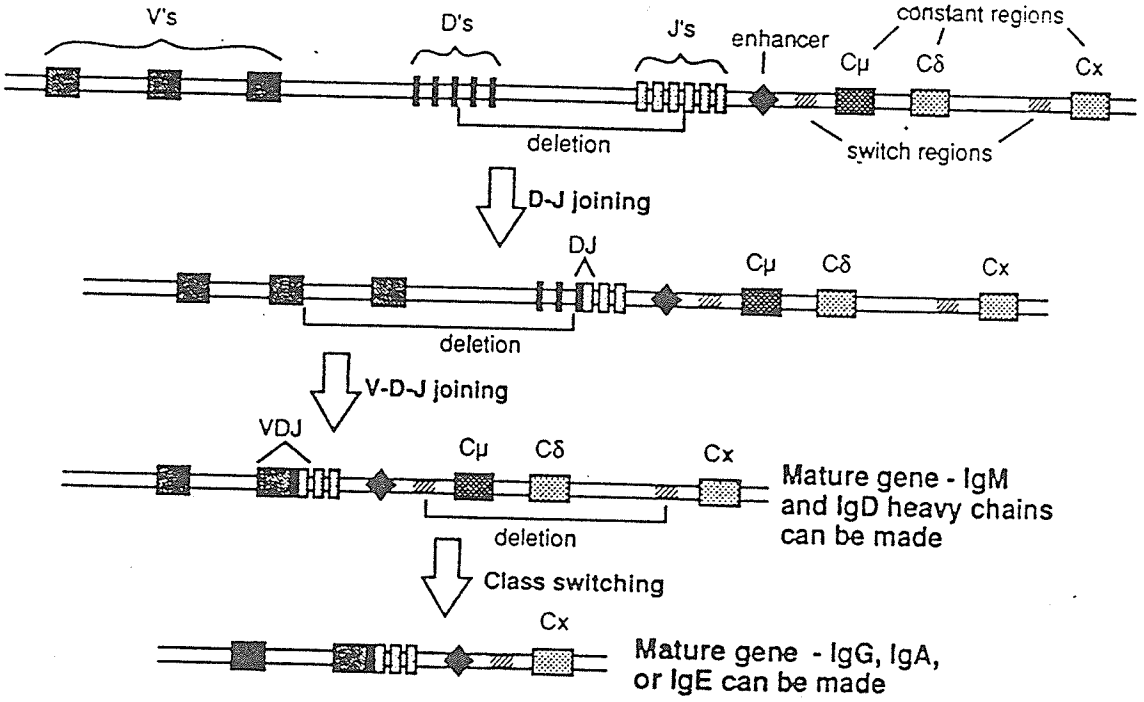
As described before, recombination of V_H, D, and J_H occurs early in B-cell differentiation. This will determine the binding specificity of the antibody.

The constant (C_H) region is important in that it mediates effector functions, such as reguired for complement fixation, or crossing the placenta.

The total size of the IgH locus has been approximated to be 2500-3000 kb (Capra and Tucker, 1989) (Figure 10).

Figure 10: The Immunoglobulin Heavy Chain Gene
Schematic representation of the IgH chain gene switching to produce the different types of immunoglobulins (McKeithan, 1990).


14
IgH


V's
D's
J's
enhancer
constant regions
Cμ
Cδ
Cx
deletion
switch regions
D-J joining
DJ
Cμ
Cδ
Cx
deletion
V-D-J joining
VDJ
Cμ
Cδ
Cx
deletion
Mature gene - IgM and IgD heavy chains can be made
Class switching
Cx
Mature gene - IgG, IgA, or IgE can be made

III. OBJECTIVES

This study involves the cytogenetic analysis of various of nonHodgkin's lymphoma (NHL). The results of the study will then be correlated with the histopathology of NHL. In situ hybridization technique will be used to study *bcl-2* gene rearrangement because the involvement of chromosome 18, specifically at 18q21, has been observed in many cases of B-cell type of nonHodgkin's lymphoma. Also the Southern blot analysis will be done on cases that do not exhibit the t(14;18) chromosomal translocation, in order to determine whether *bcl-2* rearrangement has occurred, but was not detected through the cytogenetic analysis.

IV. 1. MATERIALS AND METHODS

Materials

Lymph node biopsy specimens were received from the Department of Pathology after surgery. The *bcl-2* genomic probe was donated by Dr. Y. Tsujimoto from the Wistar Institute in Philadelphia.

The probe used in this study, was the 3.5kb , EcoRI - HindIII, *bcl-2* genomic fragment (Figure 8). This fragment is from chromosome 18 band q21.3. It is a proto-oncogene that has been observed to be involved in the translocation t(14;18)(q32;q21). The proto-oncogene, *bcl-2* gene has been inserted into the multiple cloning region of pSP65 at the EcoRI - HindIII restriction enzyme site. The major breakpoint region is located within this fragment.

IV. 1.1 Cytogenetic Analysis

Culturing Techniques

The tissue was first washed in RPMI 1640 containing antibiotics. Then using sterile scissors and forceps, the tissue was mechanically minced to achieve a single cell suspension which was then aliquoted into petri plates. To each plate 5 ml of RPMI 1640 with antibiotics and 20% fetal calf serum was added. Mitogens were not added since this would also stimulate the normal cells to divide and only the spontaneously dividing tumor cell are being examined. The cells were cultured for 24 hours at 37°C. One hour before harvesting, 0.05μg/ml of colcemid was added. Cells were then fixed in 3:1, methanol:acetic acid fix. These were then stored at 4°C overnight to allow proper fixing.

Chromosome Preparation

The next day cells were washed several times in fixative before slides were prepared. Slides were rinsed in cold distilled water before use. The suspension of fixed cells were dropped onto wet slides. The slides were dried under humid conditions.

G-banding

Slides were aged for approximately 8-10 days before G-banding. For G-banding, slides were treated with trypsin solution. Trypsin solution consisted of 0.85% saline solution with trypsin. The solution was at approximately 17°C. Metaphase cells were treated for about 4-6 seconds. The slides were then rinsed twice in 0.85% saline, then stained in 4% Giemsa for 4 minutes, and air dried. Metaphases were studied under the light microscope. Metaphses were also photographed.

Analysis of each metaphase cell was made from the photographs. Up to 5-25 metaphases were studied per patient, and this depended on how well the chromosomes banded. Karyotypes were made for each patient.

This procedure was used prior to *in situ* hybridization. Slides that were G-banded were destained and used for the *in situ* hybridization study.

Slides were also G-banded after *in situ* hybridization. After developing the slides, each slide was treated with trypsin for 5 minutes at 17°C. Slides were rinsed in normal saline and then stained in Giemsa for 5 minutes. After staining, the slides were treated with trypsin for another 5 minutes and stained again (personal communication from Dr. M. Ray).

IV. 1.2. In Situ Hybridization

The technique of in situ hybridization has been used to detect nucleic acid hybridization on cytological preparation. This technique allows for the localization of single copy gene to specific sites on the chromosomes. The DNA sequence is usually labelled with ^{3}H or ^{35}S and hybridized to metaphase spreads. After hybridization the slides are dipped in photographic emulsion. Slides are then developed after appropriate time required to expose the emulsion to radioactivity. Then the slides are stained for viewing (Figure 11).

Pretreatment of Slides

Before in situ hybridization, the slide had to be treated. Metaphase spreads were treated with pancreatic RNase A (Sigma), at a concentration of 100 $\mu g/ml$ in 2 × SSC, pH 7.0. This step is required to remove RNA which will otherwise hybridize to the probe. A coverslip was placed on each slide, and then the slides were incubated at 37°C for one hour. This was done to remove any endogenous RNA.

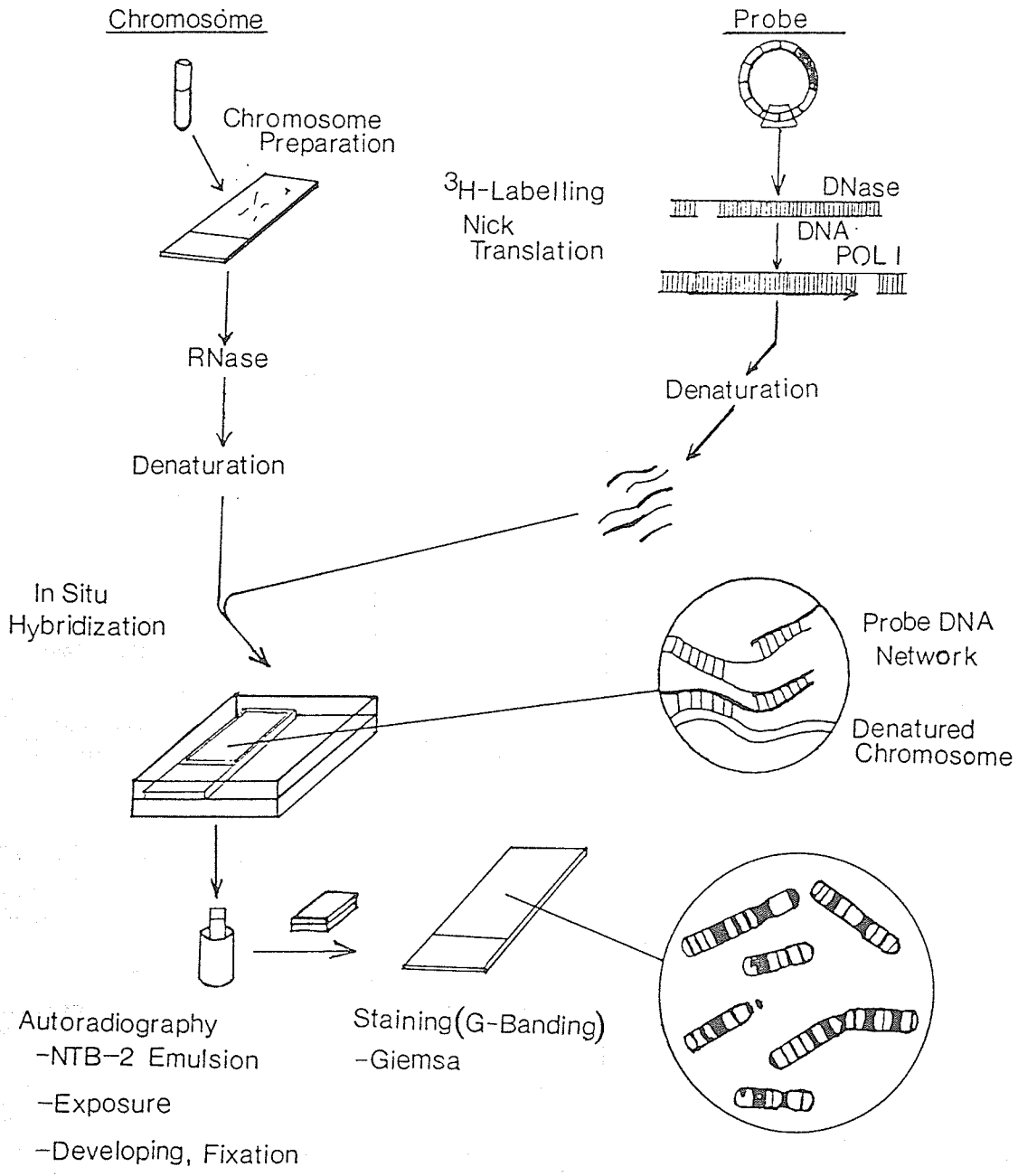
After one hour , the slides were rinsed three times in 2 × SSC, pH 7. Slides were then dehydrated in a successive alcohol series of 50%, 75%, and 95%. The slides were allowed to dry for approximately three hours.

To denature the chromosomal DNA, slides were immersed in 70% (vol/vol) deionized formamide/2 × SSC at 70°C for two minutes.

Slides were then washed in the successive solutions of ethanol as decribed above and allowed to dry overnight.

Figure 11: Schematic Representation of In Situ Hybridization (Methods in Enzymology, 1987)

SCHEMATIC REPRESENTATION OF IN SITU HYBRIDIZATION



Preparation of Probe

The plasmid with the *bcl-2* gene fragment was labelled with three types of tritiated nucleotides, [^{3}H]dATP (29 Ci/mmol), [^{3}H]dCTP (50 Ci/mmol), [^{3}H]dTTP (46 Ci/mmol) (Amersham) using the technique of random priming with oligonucleotides (Naylor et al, 1987). Specific activity for the probe used was 1.8×10^7 cpm/μg. Tritium labelled probe was washed in 70% ethanol, air dried and redissolved in hybridization buffer. Hybridization buffer consisted of 50% deionized formamide, 10% dextran sulfate, 2 × SSC, 40mM NaH_2PO_4, 0.1% SDS, and 1 × Denhardt's solution (final pH of 7.0). The probe was hybridized at a final concentration of 100 ng/ml.

The probe was denatured by heating at 70°C for 10 minutes and cooled quickly in ice. This was done prior to hybridization.

In situ Hybridization

For each slide 35 μl of radiolabelled probe was used. A coverslip was placed on top of the slide and the edges were sealed with rubber cement. The slides were then incubated in a 50% formamide/2 × SSC saturated environment. The slides were incubated for 18 hours at 42°C.

After the incubation the rubber cement was removed and the slides were dipped in 50% formamide/2 × SSC at 40°C to remove the coverslips. Next the slides were washed 3 times in 50% formamide/2 × SSC pH 7.0 at 40°C. This removes the nonspecifically bound DNA.

Slides were then washed three times for 10 minutes in 2 × SSC pH 7.0 at 40°C. After this, slides were

washed three times for another 10 minutes in 2 × SSC, but this time at room temperature.

In the next series of washes, the slides were washed for one hour in 0.1 × SSC, pH 7.0 at room temperature. Final wash was also done in 0.1 × SSC for one hour but at 4°C.

Finally the slides were dehydrated in four washes in cold (4°C) ethanol at successive concentrations of 25%, 50%, 75%, and 95% and air dried overnight.

Autoradiography

All steps were done in a dark room. Hybridized slides were dipped in Kodak emulsion that had been diluted 1:1 in distilled water. The temperature of the emulsion during dipping was 42°C.

The slides were dried for two hours in the dark. The slides were then sealed in bakelite boxes containing Drierite dessicant and exposed for 10 - 12 days. During this period the slides were kept at 4°C.

After the exposure period, slides were developed for 75 seconds in Kodak Dektol at 20°C. These were then fixed for 30 seconds in Kodak fixer diluted 3:1 in distilled water. The slides were finally rinsed in water, and air dried overnight.

Analysis of Slides

After developing the slides, each metaphase cell previously photographed after G banding were rephotographed. From these photographs, grains were counted and statistically analyzed.

Any metaphase cells having a grain or grains on or near a chromosome were photographed. The grains were counted and statistically analyzed (Morton et al., 1984).

The location of each grain was recorded.

IV. 1.3 Southern Blot Analysis

Cases that were used in the in situ study were also used for Southern blotting for confirmation of the in situ results. Cases that showed a few cells with chromosome 18 involvement were also used in this analysis.

DNA extraction and Southern blotting was performed according to standard procedure described in Maniatis (1982) but with a few modifications. DNA was digested with EcoRI restriction enzyme for 24 hrs. and run overnight on 1% agarose gel. Gel was photographed and then blotted over night. Nitro Plus 2000 (Micron Separation Inc.) was used for the Southern transfer.

Probe used for this study was the 3.5kb *bcl-2* genomic fragment received from Tsujimoto. The probe (200 ng) was then nick translated using 50 μCi of α-^{32}P-dCTP. The specific activity of the labelled probe was 2.6×10^8 cpm/μg of plasmid DNA.

The blot was incubated in prehybridization solution (50% formamide, 5 x SSC, 5 mM $NaPO_4$, pH 6.5, 250 μg/ml salmon sperm DNA, and 1 x Denhardt's Solution) overnight at 42^{o}C. The prehybridization solution was dumped out and hybridization solution was added. The hybridization solution was the same as the prehybridization solution except that it contained 10% dextran sulfate and the labelled *bcl-2* probe. The blot was then incubated overnight at 42^{o}C.

The blot was washed the next day for 5 min. in a solution of 2 x SSC and 0.5% SDS at room temperature. The second wash was also at room temperature but this time the blot was washed for 15 min. The third wash was

in 0.1 x SSC, 0.5% SDS for 2 hrs at 68^{o}C. The final wash was also at 68^{o}C, in 0.1 x SSC, 0.5% SDS but only for 30 min. The blot was then wrapped in plastic and autoradiographed overnight. The X-ray film was then developed in Kodak developer and fixer, for visualization of the exposed bands. These bands represented the site where hybridization had occurred between the *bcl-2* probe and the EcorI digested DNA in each lane.

V. Results

In this study, ten nonHodgkins lymphoma were examined cytogenetically. Two cases were probed with the *bcl-2* gene using the in situ hybridization technique. This was due to the lack of large number of cells that is required for a statistical analysis of grain distribution. These two case plus another six cases were used for the Souther blot analysis. The remaining two out of the ten cases were not used in either in situ hybridization or Souther analysis since not enough material was available. Table 2 lists the cases which have been examined.

Table 3

CASES OF NONHODGKINS LYMPHOMA

CASE NUMBER	AGE/SEX	TYPE OF NHL
1	55/F	Diffuse large cell, immunoblastic, high grade. Stage II A
2	77/M	Diffuse large noncleaved cell intermediate grade Stage IV.
3	57/M	Diffuse large non-cleaved cell. Intermediate to high grade. Stage IV.
4	41/M	Follicular small cleaved cell. Low to intermediate grade. Stage I.
5	41/M	Diffuse small lymphocytic. Low grade.
6	48/M	Diffuse large noncleaved cell. Intermediate grade.
7	87/M	Follicular large cleaved cell. Intermediate grade

Table 3-continued.

CASE NUMBER	AGE/SEX	TYPE OF NHL
8	77/M	Follicular small cleaved cell. Low grade. Stage IV.
9	57/M	Diffuse large noncleaved cell. Intermediate grade.
10	77/F	Follicular small cleaved cell. Low grade. Stage IV.

V. 1. Cytogenetic Results

Case 1 Diffuse, large cell, immunoblastic lymphoma
High grade

This 55 year old woman presented with a four month history of intermittent abdominal pain, not associated with anorexia, nausea or vomiting. A CT scanning of the abdomen showed swelling around the head of the pancreas. A follow up scan confirmed a mass confluent with the retroperitoneal lymph nodes. Needle biopsy suggested a malignant lymphoma. There was no history of fever and chills, and weight loss of 7 lbs. over the previous three months was observed.

Prior to further investigation she was presented to emergency with persistent epigastric pain. Physical examination revealed a fullness of the epigastrium. The liver edge was palpable and there was no splenomegaly or lymphadenopathy. She proceeded to laparotomy with extensive intra-abdominal malignant lymphoma (stage II) immunoblastic, high grade with B-cell phenotype (Working Formulation) was found. She was treated with MACOP-B regimen with excellent response to chemotherapy, and remains well 6 months post-treatment with no evidence of lymphoma.

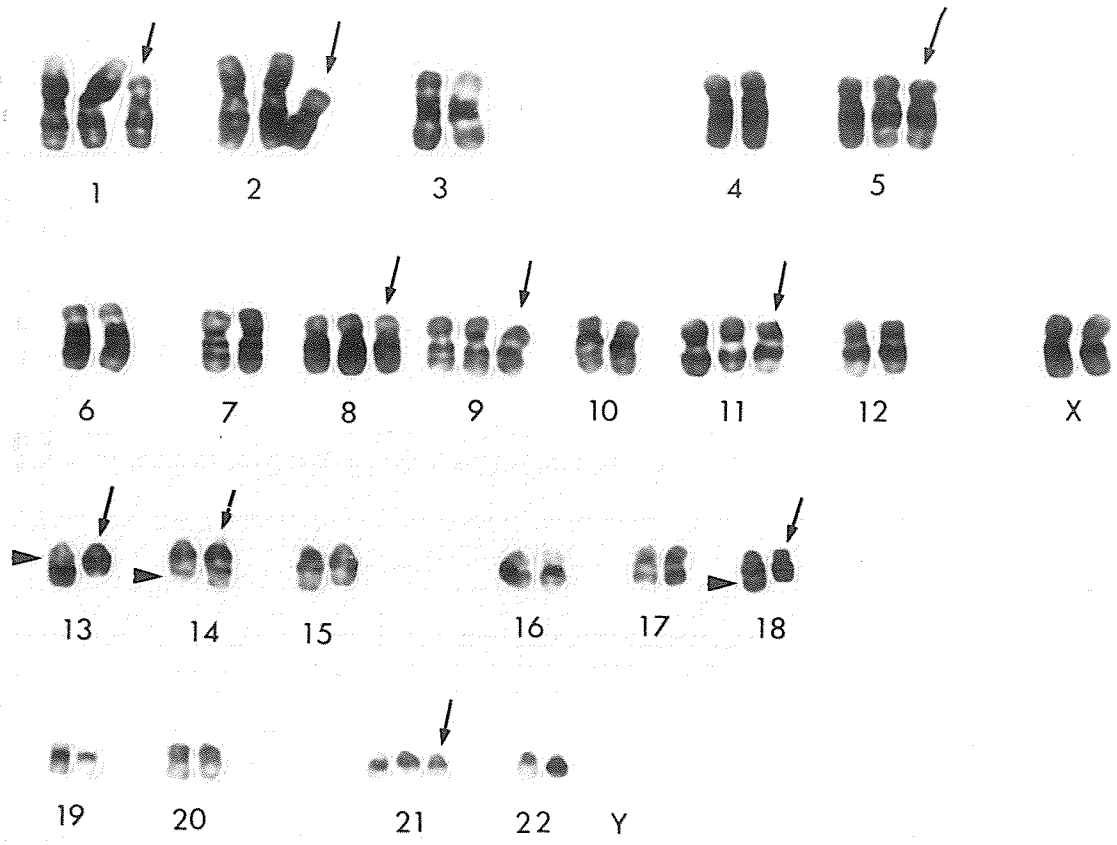
Cytogenetic analysis indicate at least two clones of cells. Thirty-one cells were analyzed. Combined karyotype of the patient in 53, XX,+1p-,+5,+7,+9, +11,int del(13)(q13-q14),t(14;18)(q32;q21),+21,+M_1,+M_2,+M_3 (Figure 12).

This case exhibits a translocation t(14;18)(q32;q21) in 13 out of 31 cells examined. Another 13 cells show a

14q+ marker chromosome, but the donor chromosome cannot be identified using standard G-banding techniques. Also this particular case shows an int del(13)(q13q14) as well as trisomy 7, 8, 9, 11, and 21.

Each cell exhibits multiple abnormalities. The chromosome ploidy of this case ranges from 49-55.

Figure 12: Representative karyotype of diffuse large cell immunoblastic lymphoma, high grade.
53,XX,+1p-,+5,+7,+8,+9,+11,del(13)(q13q14),
t(14;18)(q32;q21),+21.
Arrows indicate trisomies and arrow heads show chromosomal regions involved in translocations and deletions.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21
22
Y

Case 2 Diffuse, large, noncleaved cell lymphoma
Intermediate grade

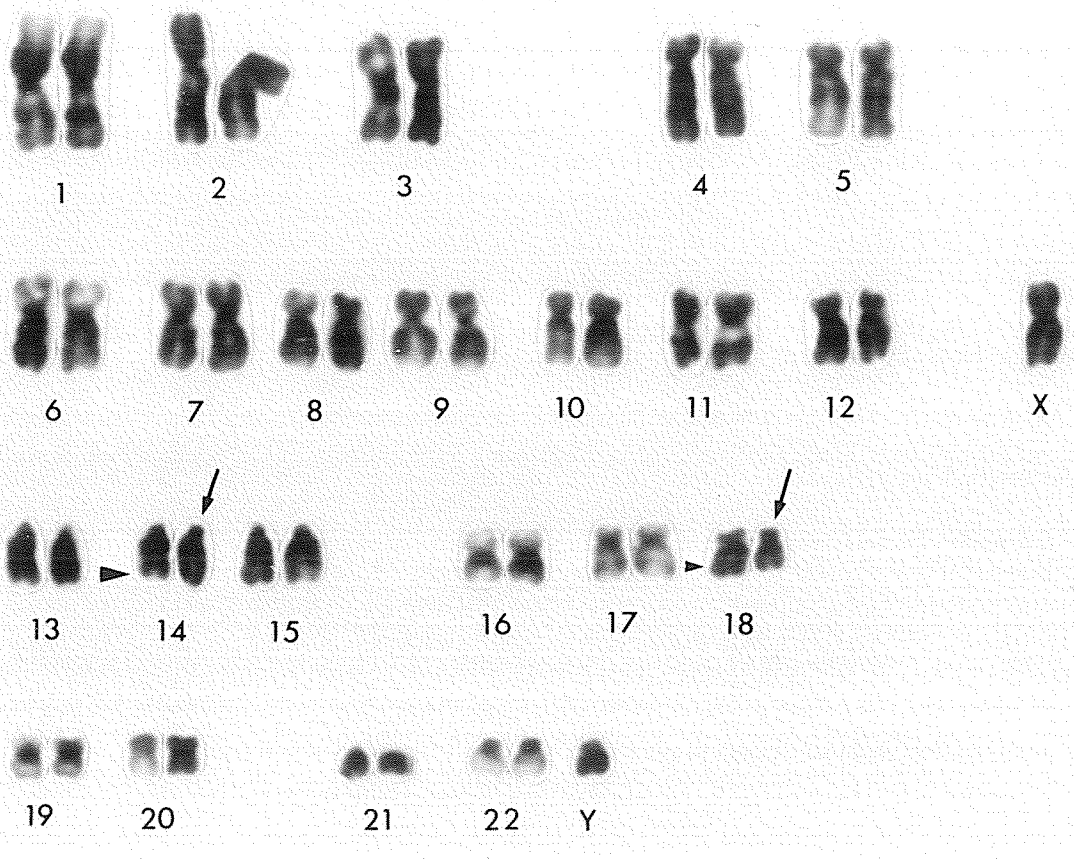
On October, 1988, a 77 year old man presented with an abdominal mass and generalized lymphadenopathy. Biopsy specimen from right axillary node showed large, noncleaved cell, B-phenotype, intermediate grade lymphoma. He had weight loss and ascites.

He was treated with chemotherapy with C-MOPP. There was dramatic reduction of abdominal masses.

He was readmitted in June of 1989 with fever and urinary tract infection. He was treated with antibiotics. CT scan showed increase in lymph node size and was treated with chemotherapy but continued to deteriorate. He died August, 1989.

Cytogenetic ananlysis revealed the presence of a 14q+ marker chromosome. The donor chromosome could not be identified accurately. It may possibly be from chromosome 18 since a few cells (4/11 cells) do have an 18q-. Nine cells had the 14q+ marker chomosome. The representative karyotype for this case is 46, XY, 14q+, 18q- (Figure 13).

Figure 13: Diffuse large noncleaved cell lymphoma. Its representative karyotype is 46,XY,14q+,18q-. Arrows indicate chromosomes involved in translocations.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22
Y

Case 3 Diffuse, large, noncleaved cell lymphoma
Intermediate to high grade

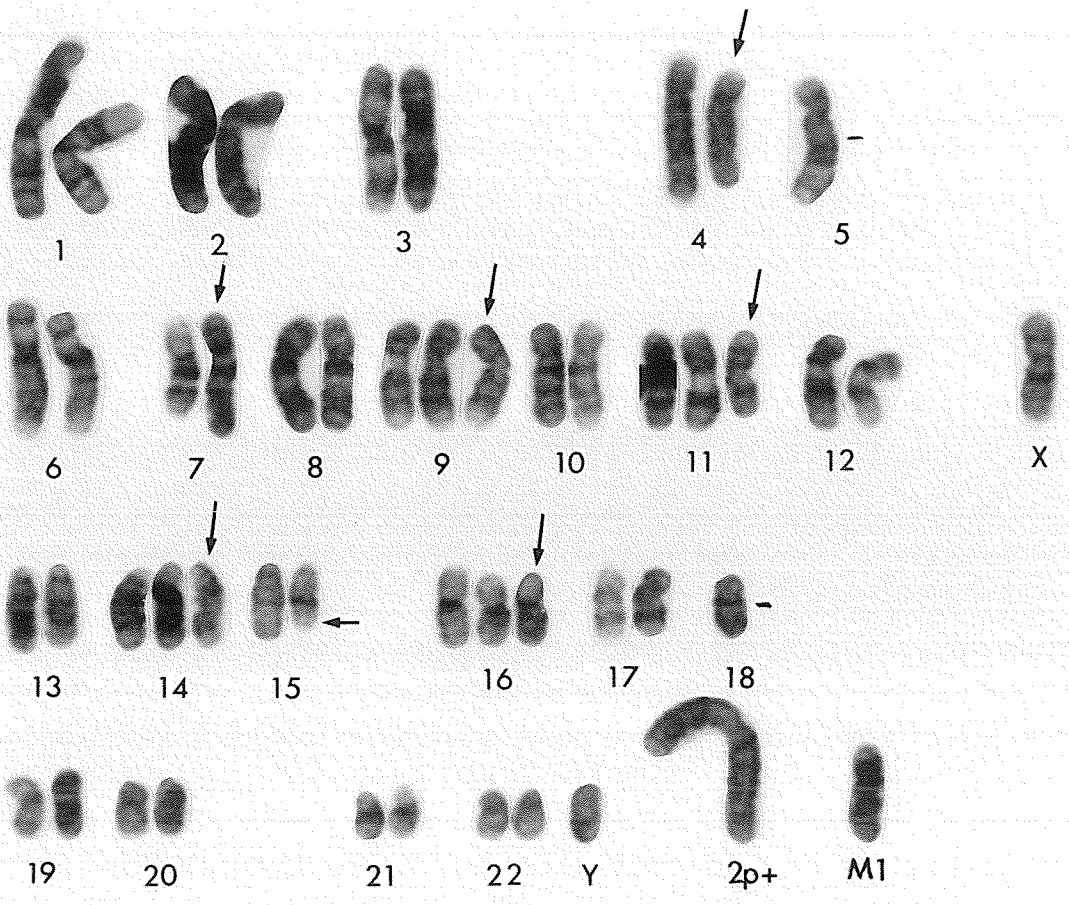
This 57 year old individual presented with increasing gentle and dull abnominal pain. By CT scan and ultrasound the presence of lymphoma was indicated. This person was diagnosed as having malignant lymphonma, diffuse large, noncleaved cell with immunoblasts, intermediate to high grade (WF) (with B-cell phenotype). Right hemicholectomy and resection of mesenteric lymphoma was performed but not all of the lymphomas was resected. Bone marrow was negative for tumor.

Sixteen metaphase spreads were analyzed from this patient. Each cell exhibited various chromosomal aberrations. The ploidy of the cells examined ranged from 41-52. Cells having 50 chromosomes were observed most often.

Through cytogenetic analysis, a karyotype of the patient has been determined to be 50, XY, 2p+,-5,+int del(11)(q14),+14,del(15)(q25qter),+16,-18,+M_1 (Figure 14).

In this case of diffuse, large noncleaved cell lymphoma, int del(11)(q14) is observed predominantly. Next, common chromosomal abnormality observed is the int del(13)(q13q14). Few cells do exhibit a 14q+ marker chromosome. Though 18q- is observed in two cells it cannot be said that the 14q+ is a t(14;18) chromosomal translocation. The extra chromosomal material can be from the another chromosome.

Figure 14: Diffuse, large noncleaved cell lymphoma with a representative karyotype of 50,XY,2p+,-5, +9,+11,+14,del(15)(q25),+16,-18,+M1. Arrows indicate chomosomes involved.


1
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12
X
13
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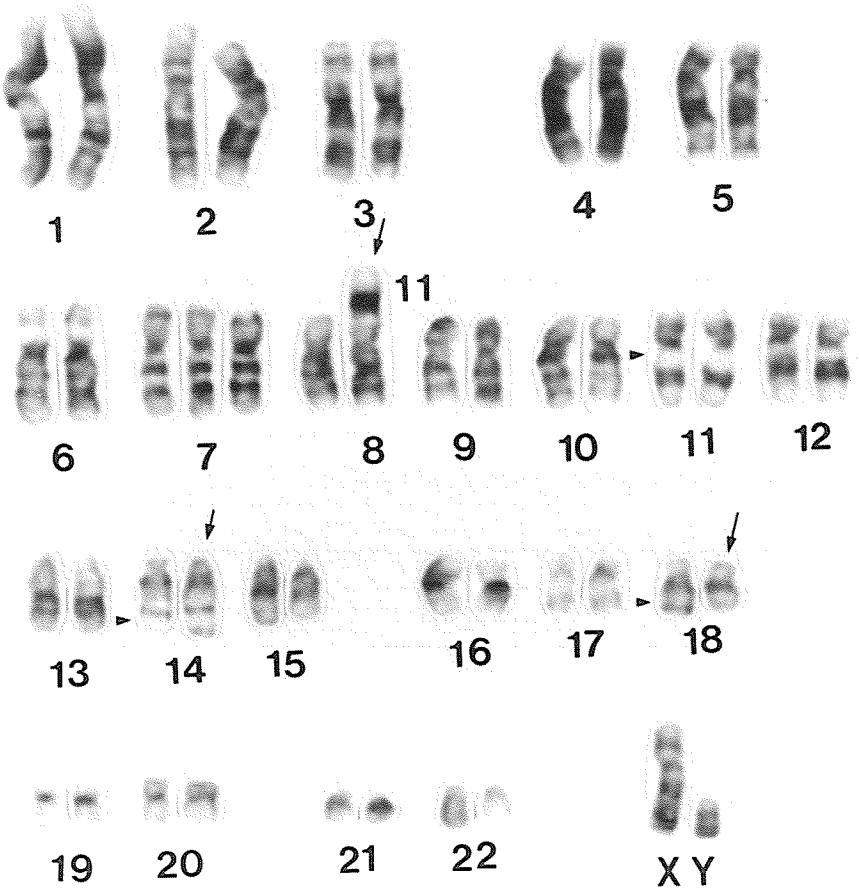
16
17
18
19
20
21
22
Y
2p+
M1

Case 4 Follicular small cleaved cell lymphoma
Low to intermediate grade

Our cytogenetic study was on a 41-year old male who represented with an 18-week history of left cervical lymphadenopathy unresponsive to antibiotics. He suffered discomfort on opening his mouth but there was no dysphagia. He was a heavy drinker and smoker, but was other wise healthy. Physical examination revealed a 6cm mass in the left upper neck. There was no lymphadenopathy or hepatosplenomegaly. Fluoroscopy was negative. Excision biopsy of the mass confirmed a malignant lymphoma, follicular small cleaved cell, low to intermediate grade. Staging procedures chest X-rays, abdominal CAT scan, bilateral bone marrow aspirates and biopsies were negative. He was treated with local radiotherapy to the involved neck nodes and remains well.

In this particular case of follicular, small cleaved cell lymphoma, the t(14;18)(q32;q21) chromosomal translocation appears to be the major chromosomal aberration. The presence of t(8;11)(p21;q13) has been detected in 24 of the 26 cells analyzed. Two of the cells did not have the t(8;11). Trisomy 7 appears in 17 cells which also exhibit the t(8;11) and t(14;18). Losses of chromosome 8 and chromosome X are seen in 14 and 9 cells, respectively. Nine out of 26 cell have all of the above mentioned chromosomal aberration (+7, -8, -X, t(8;11), t(14;18)). The nodal number ranged from 46-48 chromosomes. Various marker chromosomes were also present (Figure 15).

Figure 15: Represented karyotype 48,Y,-X,+7,-8, t(8;11)(p21;q13),t(14;18)(q32;q21) of a case of follicular small cleaved cell lymphoma. Arrows indicate chromosomes involved in translocations.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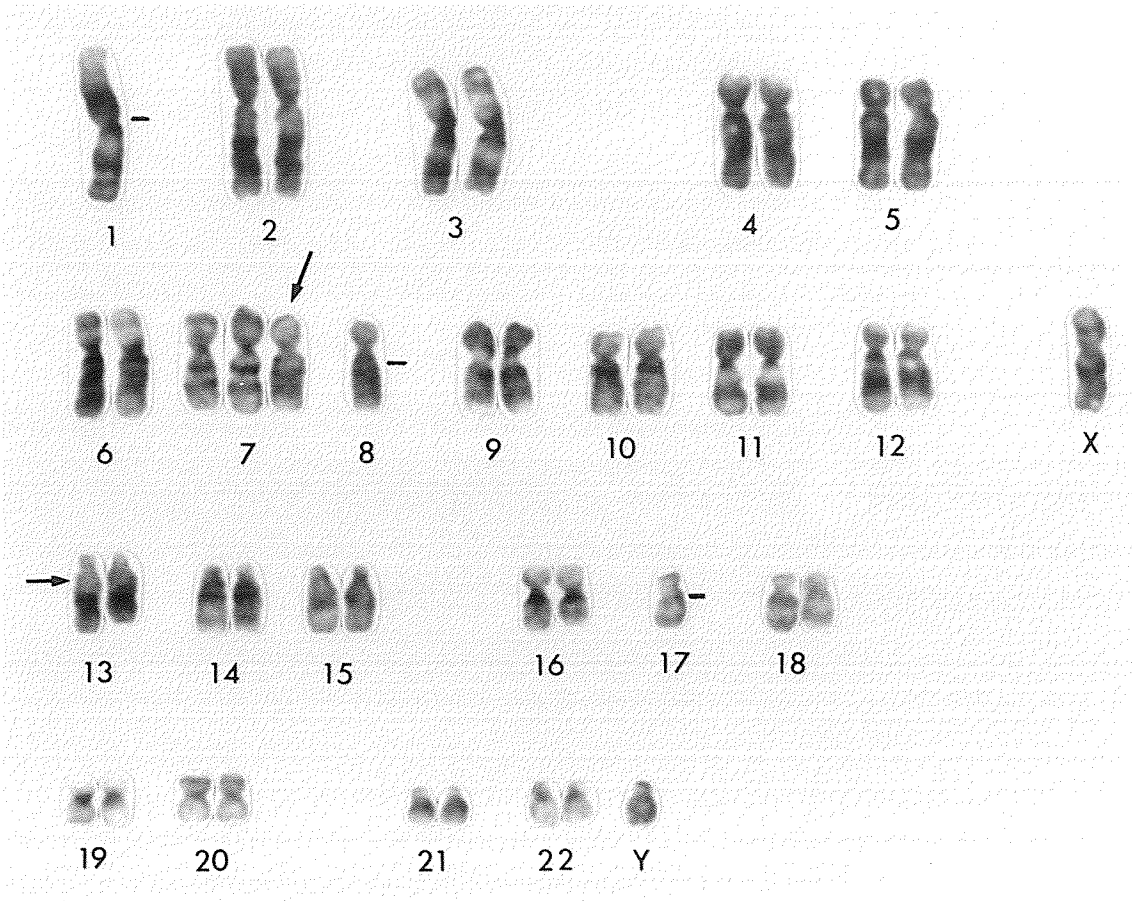
Case 5. Diffuse small lymphocytic lymphoma
Low grade

In October, 1987, this 41 year old man was admitted for excision of a lymph node from the left axilla. He was diagnosed as having diffuse small lymphocytic lymphoma with plasmacytoid features, low grade (B-cell phenotype). Large cell population was less than 20%.

Cytogenetic analysis showed no consistent chromosomal abnormalities. The significant observation is that the loss of chromosomes was detected more often in this case, but overall the nodal number was 46 (Figure 16).

Figure 16: A case of small lymphocytic lymphoma with a represented karyotype of 44,XY,+7,-1,-8,-17, del(13)(q13;q14).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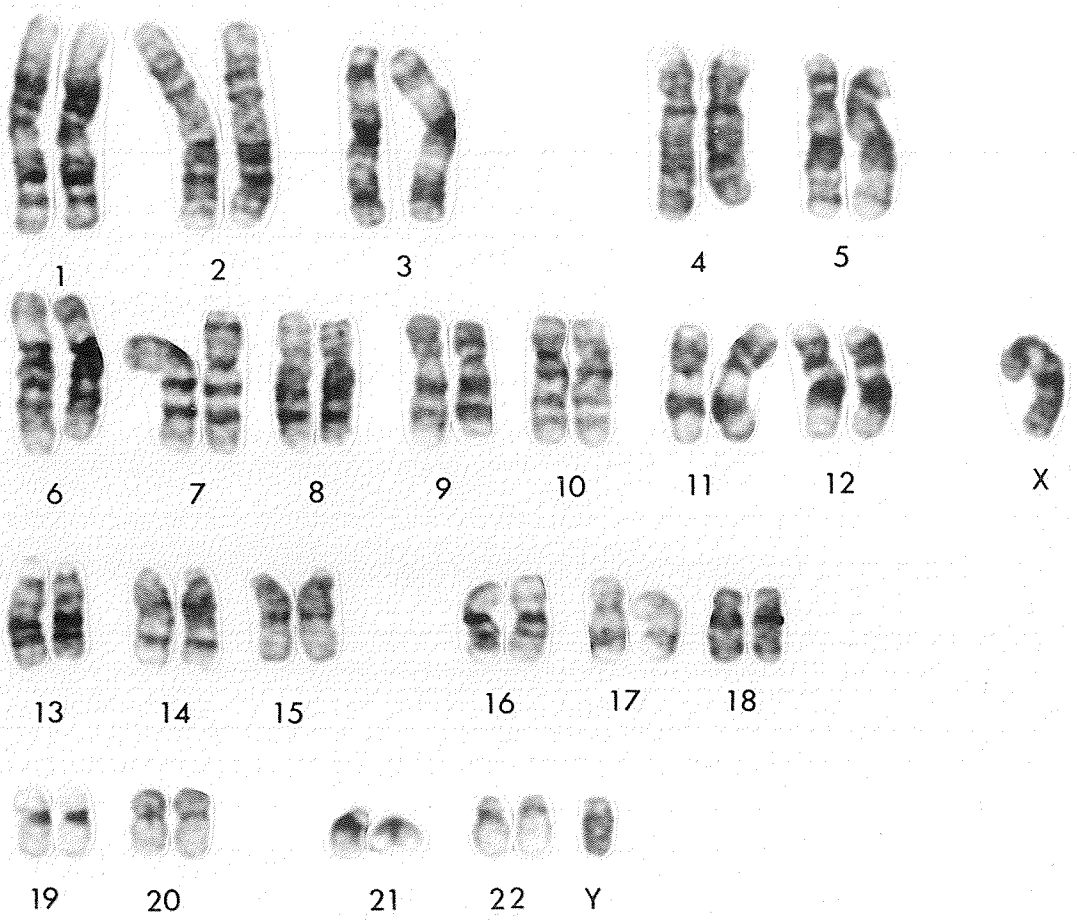
Case 6 Diffuse large noncleaved cell lymphoma
Intermediate grade

This 48 year old man presented with lymphadenopathy in the left upper cheek, but otherwise was asymptomatic. He was diagnosed to have diffuse, large noncleaved cell lymphoma of intermediate grade. He smoked half a pack of cigarettes a day for 15 years and quit in 1972.

Cytogenetic analysis showed multiple chromosomal abnormalities. These chromosomal abnormalities were not consistent and varied from cell to cell. Three out of 14 cells did show del(13q14) and 14q+ but not in the same three cells. Also various marker chromosomes were also present. The presence of normal cell was also quite prevelant (Figure 17).

Figure 17: A diffuse large noncleaved cell lymphoma having a representative karyotype of 46,XY.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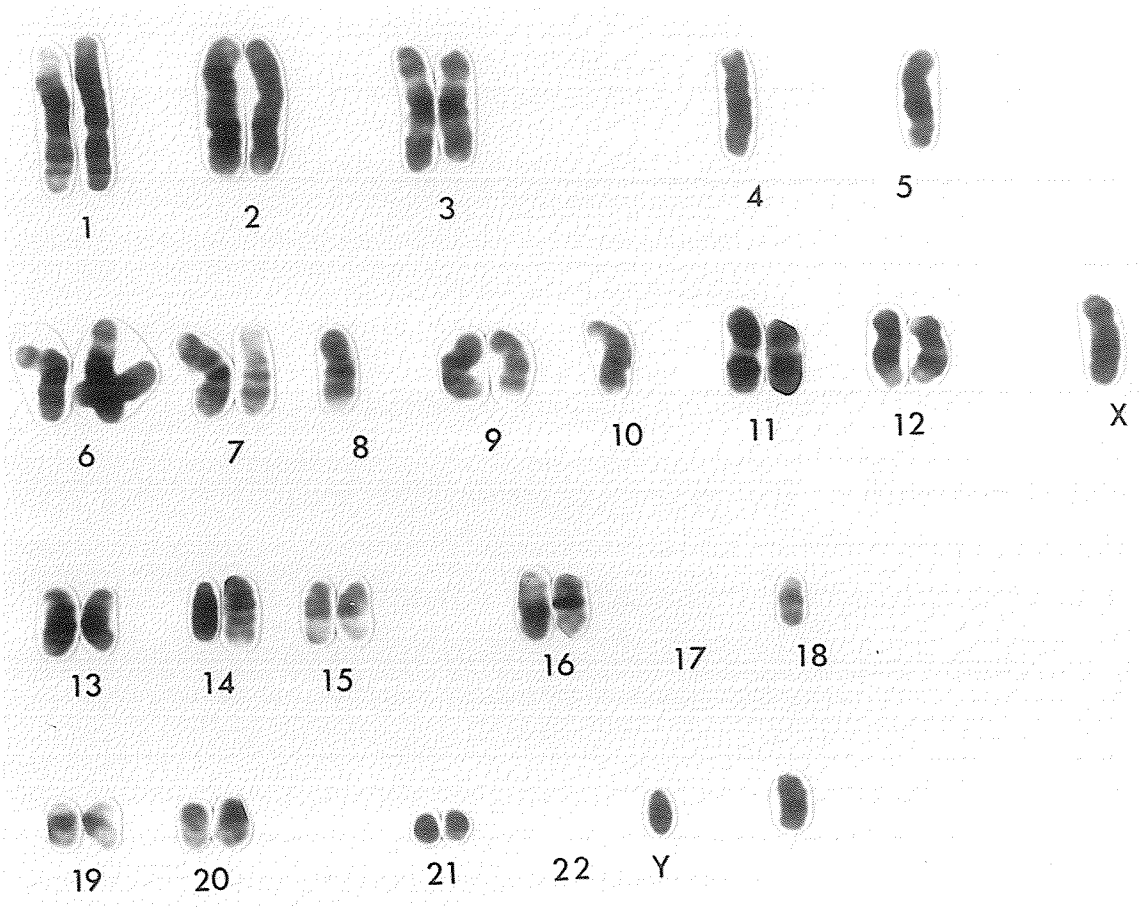
Case 7. Follicular large cleaved cell lympohoma Intermediate grade.

This man in Nov. 1987, presented with an anterior leg lesion. He was diagnosaed to have malignant lymphoma, follicular large cleaved cell with diffuse areas (B-cell phenotype).

This particular case had various random chromosomal abnormalities. The presence of 1q+ is observed in 4 cells out of 8 cells analyzed (Figure 18).

Figure 18: Represantative karyotype (partial)
39,XY,-4,-5,-8,14q+,-17,-17,-18,-22,-22,
+M_1,+M_2.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Case 8. Follicular small cleaved cell lymphoma
Low grade

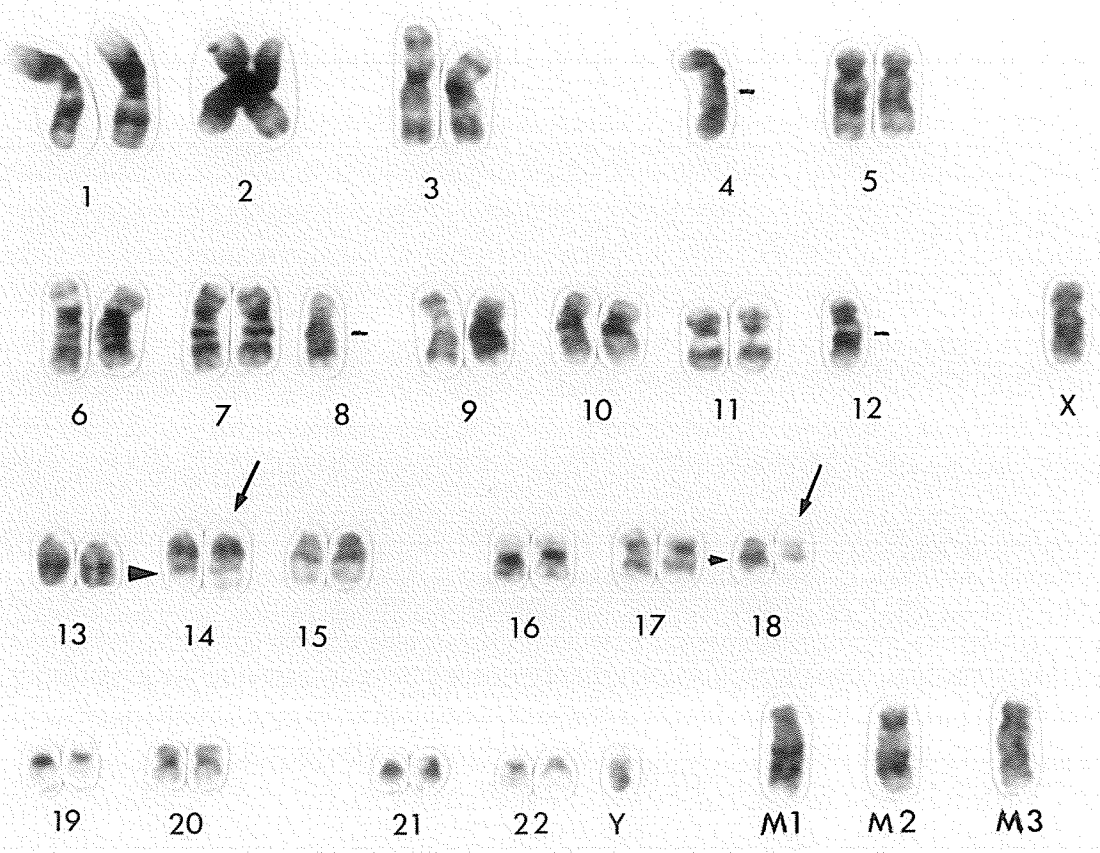
Seventy-seven year old man presented with weight loss and pleural mass and large left pelvic mass. Biopsy of mass showed follicular small cleaved cell poorly differentiated lymphocytic lymphoma. In 1981, he was diagnosed to having low grade lymphoma involving the bone marrow and was treated with chlorambucil.

In September, 1988, he was treated with C-MOPP chemotherapy. The CT scan showed no evidence of tumor.

In December, 1988, a tumor involving the spinal cord was discovered and he was treated with radiotherapy. He later died from pneumonia.

As expected, cytogenetic analysis revealed the presence of the t(14;18) chromosomal translocation. Its representative karyotype is 46,XY,-4,-8,-12, t(14;18)(q32;q21), M_1, M_2, M_3 (Figure 19).

Figure 19: Representative karyotype of a case of follicular small cleaved cell lymphoma. 46,XY,-4,-8,-12,t(14;18),+M1,+M2,+M3.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Y
M1
M2
M3

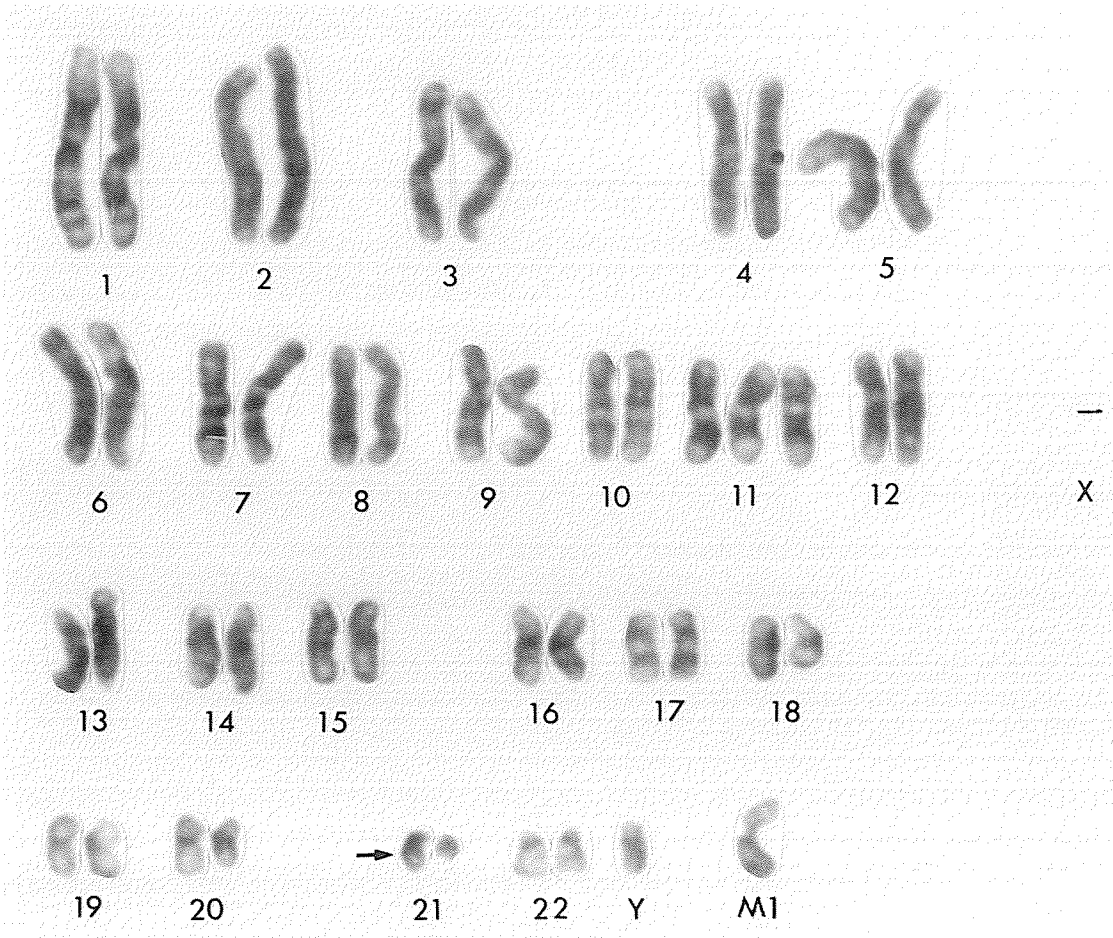
Case 9. Diffuse large noncleaved cell lymphoma Intermediate grade.

This 57 year old male has a 2 year history of preauricular mass. No nodes are involved and there was no organomegaly.

In 1987 he presented with a 5cm x 3cm mass. He had matted neck nodes and large tonsils. He had no fever, night sweats, or loss of appetite. This man was diagnosed to have diffuse large noncleaved cell lymphoma, intermediate grade (B-cell phenotype). One brother has leukemia.

Cytogenetic analysis showed multiple chromosomal abnormalities. The representative karyotype is 47, Y, -X,-11,14q+,18q-,del(21)(q22),+M (Figure 20).

Figure 20: A case of diffuse large noncleaved cell lymphoma with a representative karyotyope 47,Y,+11,14q+,18q-,del(21)(q22),+M_1.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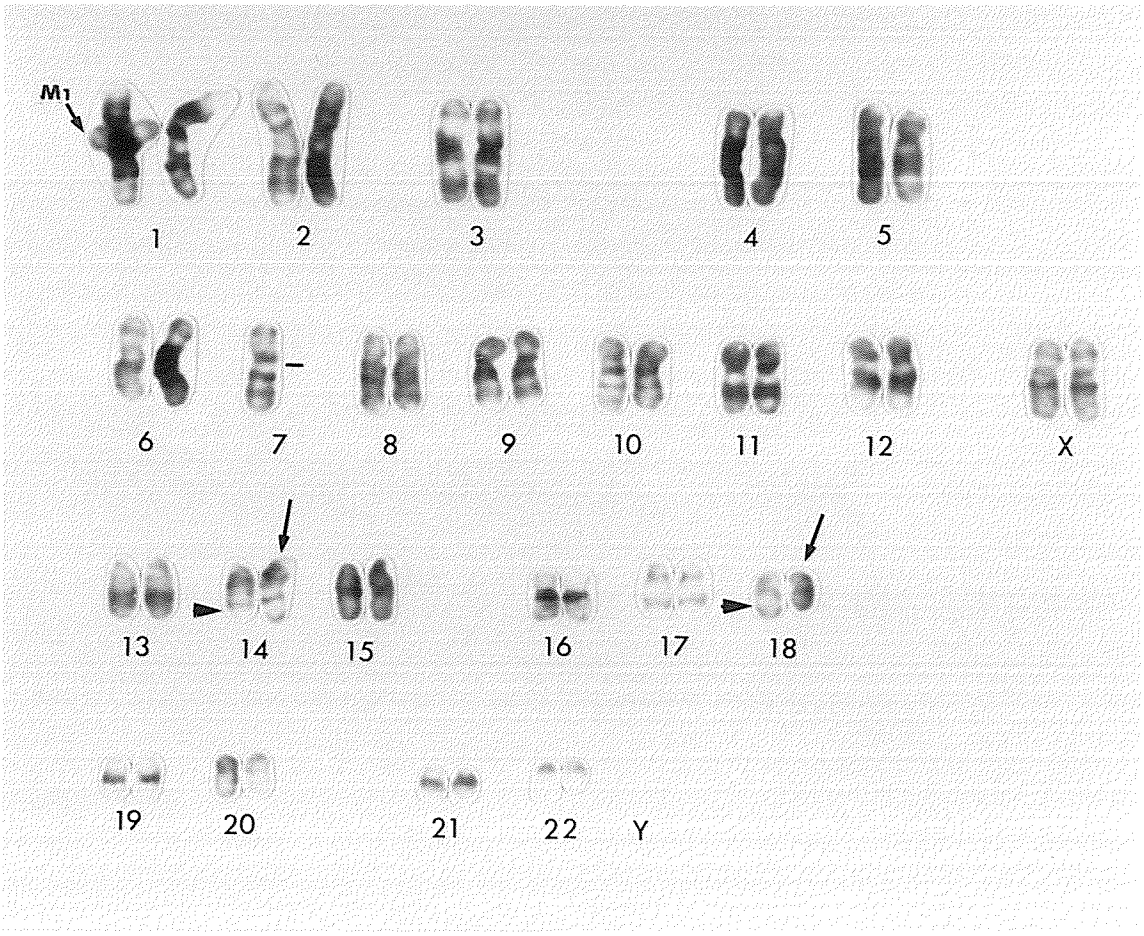
M1

Case 10. Follicular small cleaved cell lymohoma
Low grade

Sevety-seven year old woman presented with metastatic breast carcinoma with abdominal lymphadenopathy, and bilateral pleural effusion. Pleural fluid contained abnormal lymphocytes, some of which were cleaved - suspicious but not diagnostic of lymphoma. There was no tumor present in bone marrow aspirates and biopsies. She deteriorated further while in the process of treatment for NHL.

Along with various random chromosomal abnormalities, cytogenetic analysis revealed the expected t(14;18) chromosomal abnormality (Figure 21).

Figure 21: Follicular small cleaved cell lymphoma having a representative karyotype 46,XX,-7,t(14;18)(q32;q21),+M1.


M1
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Y

In Situ Hybridization Results

In situ hybridization was performed using the *bcl-2* probe. Case 1 and case 10 exhibited accumulation on the 14q+ marker chromosome. Due to uneven spreading of emulsion on some of these slides, the analysis of some metaphase spreads was not possible since the thickness of the emulsion did not allow for the banding and staining of the chromosomes. The best spreads showing grains were photographed. The prints were analyzed. Each grain was counted and the location of the grains was recorded. The frequency of a grain appearing on the 14q+ chromosome was determined.

Metaphase spreads obtained from peripheral blood lymphocytes (PBL) was used as a control (Figure 22). A total of 9 grain (9/20, 45%) ($P < 10^{-19}$) were observed on chromosome 18q21 (Figure 23).

In case 1, the case of diffuse large cell, immunoblastic lymphoma, *bcl-2* gene movement was detected (Figure 24). A total of six grains (6.96%) ($P < 10^{-9}$) were observed on the 14q+ marker chromosome (Figure 25). Also another cell showed grain distribution on chromosome band 18q21 ($P < 10^{-7}$) and in these cells the 14q+ did not exhibit any grains. This appears to support the cytogenetic analysis where some cells exhibit the t(14;18) chromosomal translocation and the other cells show a 14q+ and the chromosome 18 appears normal.

Case 10 is a follicular small cleaved cell lymphoma. This case also exhibited *bcl-2* gene movement to chromosome 14q32 (Figure 26). A total of 13 grains (31%) were observed on 14q32, ($P < 10^{-19}$) which is the site of the IgH locus (Figure 27).

Figure 22: Photograph of metaphase spread from PBL hybridized with *bcl-2* gene fragment. Arrow indicates chromosome 18 with a grain.

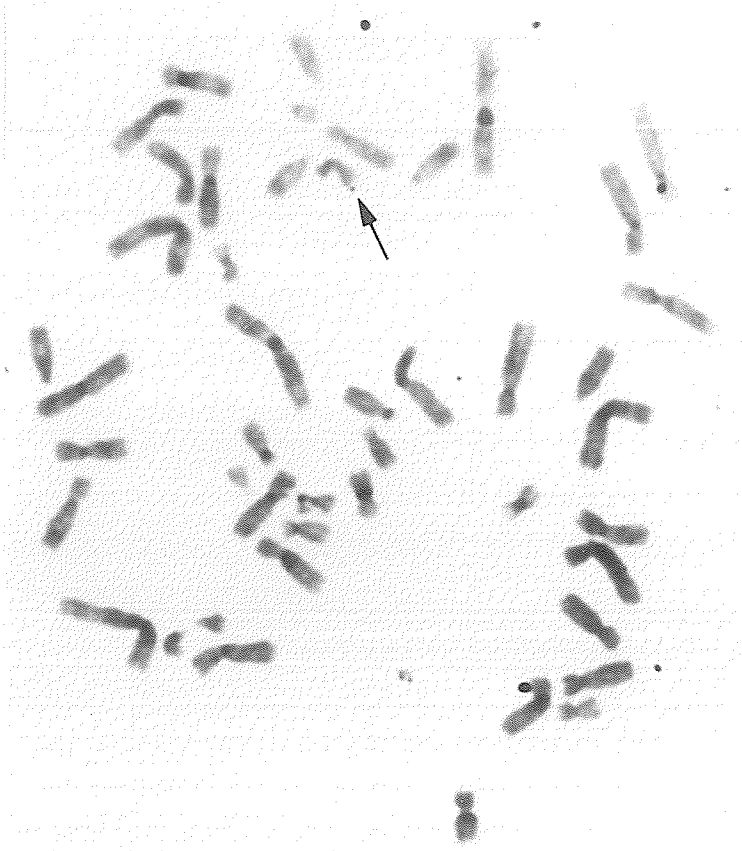
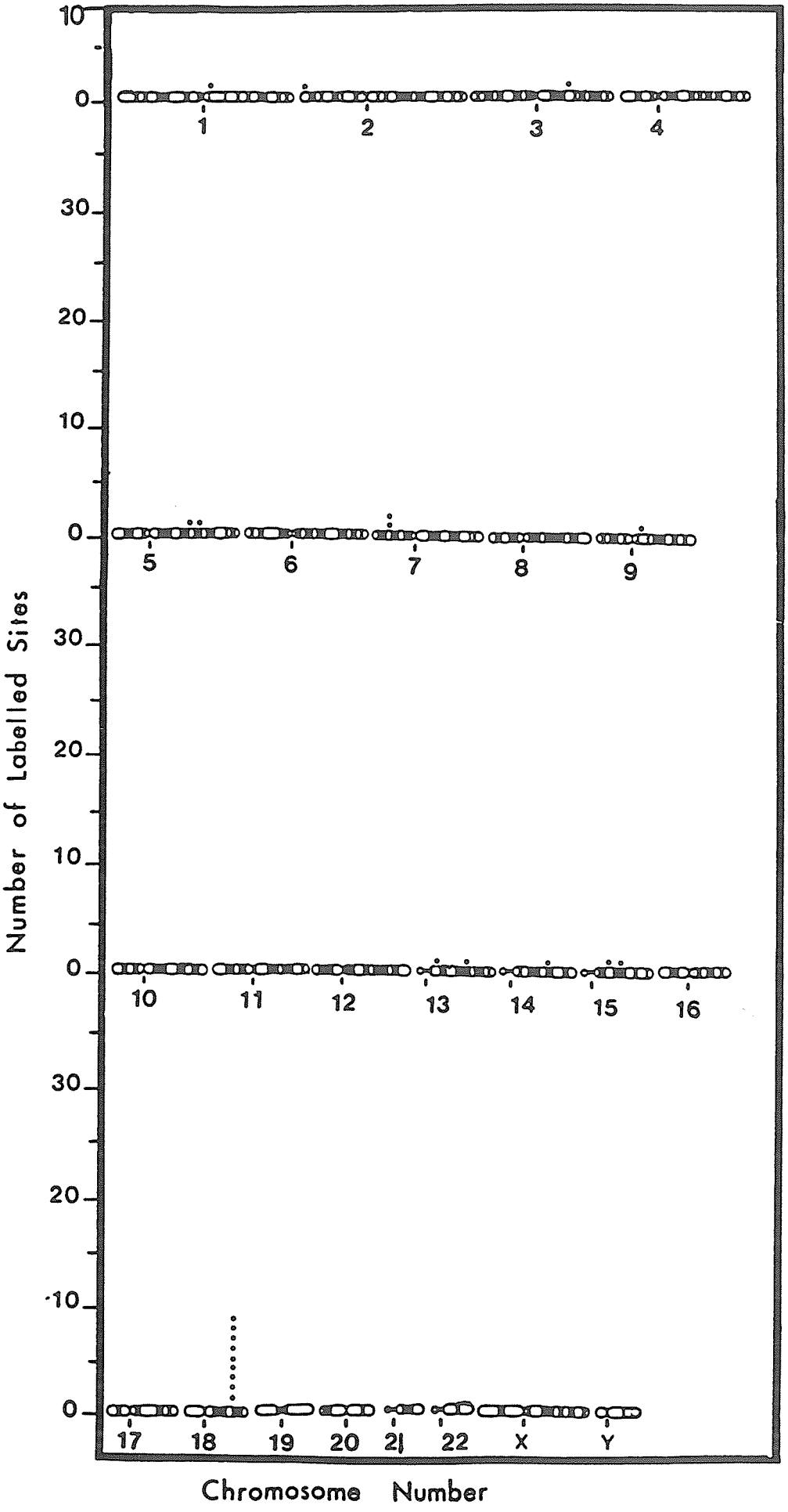


Figure 23: Histogram showing the distribution of grains from 20 metaphase spreads. A highly significant grain deposition can be seen at 18q21.


Number of Labelled Sites
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X
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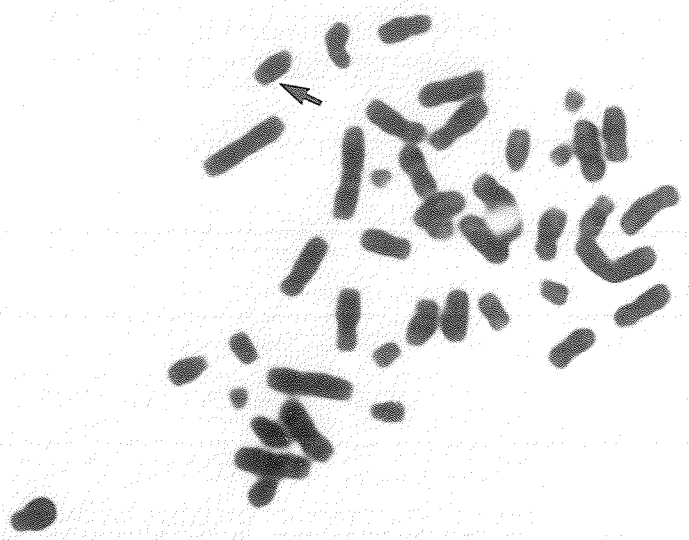
Chromosome Number

Figure 24a: G-banded metaphase spread of immunoblastic lymphoma. The arrow is indicating the 14q+.

Figure 24b: Photograph of same spread after in situ hybridization. The arrow is pointing at the site of hybridization.

a



b

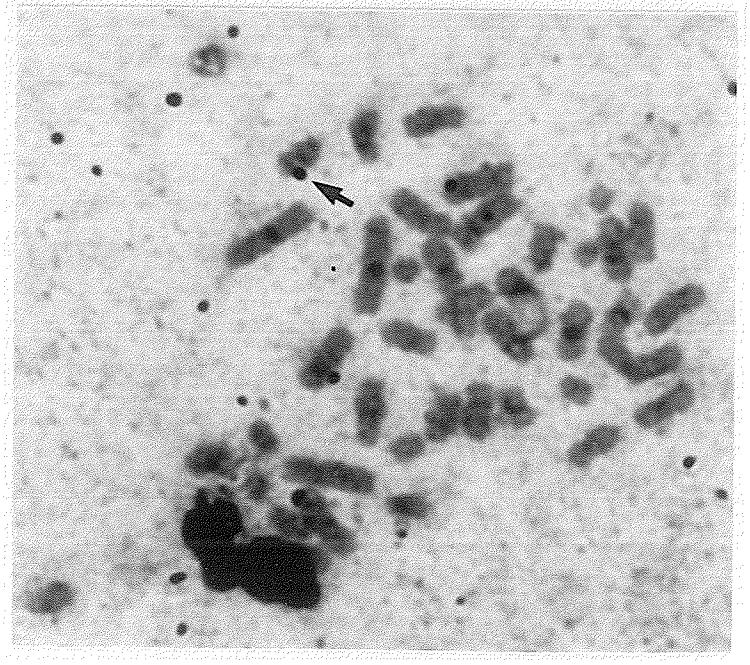
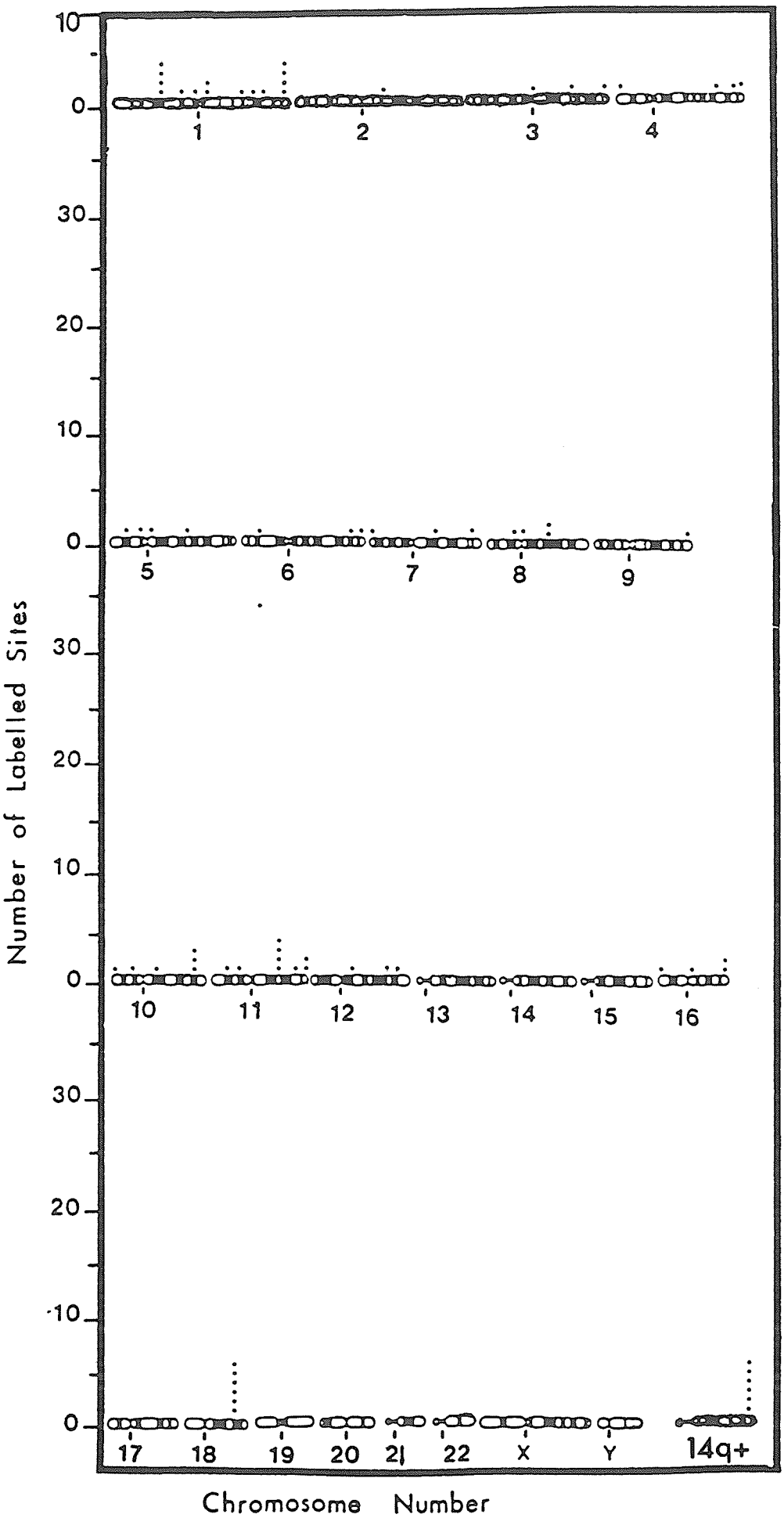


Figure 25: Histogram showing grain distribution of both chromosome 14q+ and normal chromosome 18 in immunoblastic lymphoma.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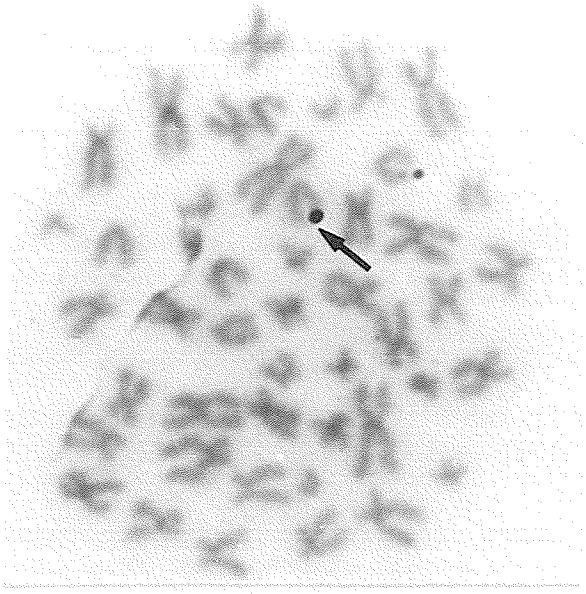
X
Y
14q+
Chromosome Number

Figure 26a: Photograph of a metaphase spread from case 10, follicular small cleaved cell lymphoma. The arrow indicates the site of hybridization.

Figure 26b: This is another metaphase spread of the same case that has been G-banded after in situ hybrization. The arrow indicates the site of hybridization.

a



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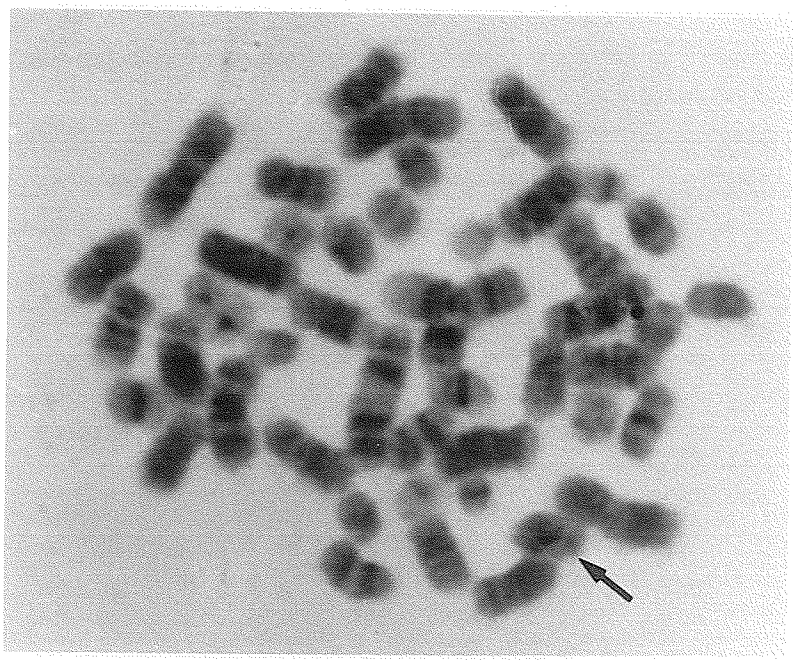
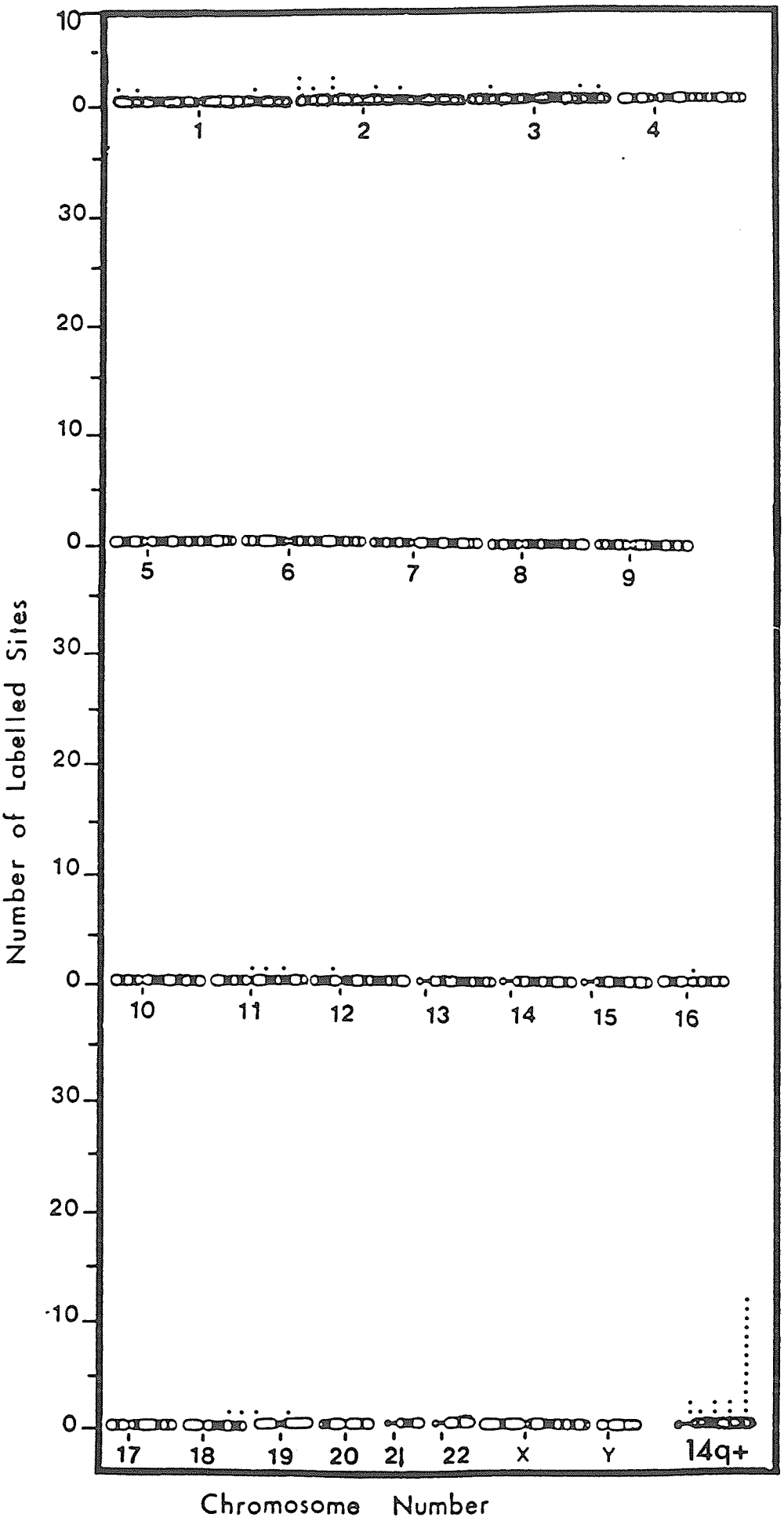


Figure 27: Histogram showing grain distribution of 36 metaphase spreads from case 10.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X
Y
14q+
Number of Labelled Sites
Chromosome Number

Southern Blot Analysis of Eight Cases of NHL

Five cases of diffuse lymphomas and 3 cases of follicular lymphomas have been probed with the *bcl-2* gene fragment (Figure 8, pg 34). Only 8 cases were studied in this manner since material for the other 2 cases were not available at the time.

The first lane (A) contains the $\lambda^{HindIII}$ marker DNA (Figure 28). This was used to size the fragment of DNA detected with the *bcl-2* probe.

Lane (1) contains DNA from case 1 (diffuse large cell, immunoblastic lymphoma, high grade). Three bands were detected with this probe. The germline band being approximately 5.7kb. Since three bands were detected it is most probable that the breakpoint occurred at the major breakpoint region which is found within the probe used in this study.

Case 2 seen in the next lane is the case of diffuse large noncleaved cell lymphoma. This lane also contains 3 bands. Germline band is located around the 5.7kb mark.

Case 3 on the next lane is a diffuse large noncleaved cell lymphoma. It also showed 3 bands indicating *bcl-2* gene rearrangement.

Case 4 is a case of follicular , small cleaved cell lymphoma. Two low molecular weight bands were detected.

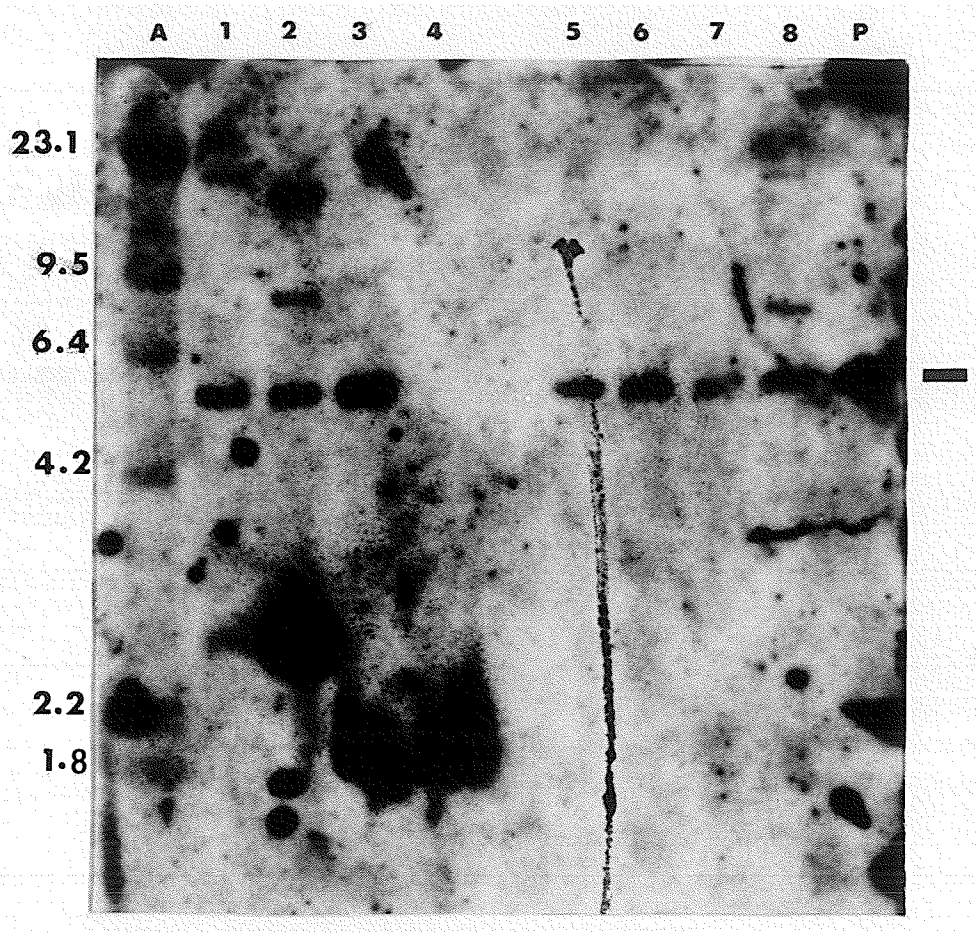
Cases 5, 6 and 7 exhibited no *bcl-2* gene rearrangement. Only the single germline band was detected.

Case 8 which is another case of follicular small cleaved cell lymphoma, exhibited 3 distinct band. This demonstrated that a *bcl-2* gene rearrangement has occurred.

Lane (P) contains DNA from peripheral blood

lymphocytes from an unaffected individual. No rearrangement is detected. Only the germline band is observed as expected.

Figure 28: Southern blot of cases 1 - 8. The $\lambda^{HindIII}$ is on the first lane on the left, lane (A).
Cases 1 - 8.
Lane (P) on the right contains DNA from PBL.
Bar indicates germline band.
Arrows indicate rearranged fragments.
The genomic DNA was digested with restriction enzyme EcorI.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VI. DISCUSSION

In this study all cases showed multiple chromosomal aberrations. From this study it is not possible to say definitively that these abnormalities are specific for each histological type of nonHodgkins lymphoma (NHL). What it does indicate is that the translocation involving chromosome 14 and chromosome 18 may play a major role in many cases of B-cell type of NHL.

Diffuse large cell, immunoblastic lymphoma

Case 1 which is a case of diffuse large cell, immunoblastic lymphoma, high grade, shows the distinct t(14;18)(q32;q21) chromosomal translocation. This is demonstrated in the in situ hybridization study where the *bcl-2* gene has moved to chromosome 14. Also the Southern analysis indicates that *bcl-2* gene rearrangement has occurred. Since the *bcl-2* gene probe used in this study does contain the major breakpoint region, it is most probable that in this case the rearrangement has occurred within this region.

For this particular case, it is possible that two clonal population of cells are involved. This is indicated through the cytogenetic analysis. Cytogenetic analysis indicates that 40% of cells analyzed have the t(14;18) chromosomal translocation. Another 40% of cells do exhibit the 14q+ marker chromosome, but appear to have the two normal chromosome 18. This suggests that the extra chromosomal material observed on chromosome 14 is derived from a chromosome other than chromosome 18. The remaining 20% of cells could not be analyzed thoroughly due to the poor quality of the chromosomes.

Case 1 also has several other consistent chromosomal

abnormalities. These include trisomy 1 with a partial deletion of the short arm, trisomy 5, 7, 9, 11 and 21. There is alo an interstitial deletion of chromosome 13 at band 13q14.

The deletion on chromosome 13 occurs at the site where a particular anti-oncogene, the RB1 is located. This site has been observed to be associated with the development of retinoblastoma (Rowley, 1990). Therefore, it is possible that loss of this gene in this particular case of immunoblastic lymphoma may lead to the progression of the disease, since this gene is considered to be a tumor suppressor gene.

Also the presence of trisomy 7 may also cause the disease to become an aggressive one. It has been shown by Kaprowski et al. (1985) that the expression of the receptor for epidermal growth factor correlates with increased dosage of chromosome 7 in malignant melanoma. With extra copies of chromosome 7, it is possible that these cells have acquired a growth advantage which allows them to progress towards a more high grade type. Therefore it is possible that the presence of other extra chromosomes may have a similar role in tumorigenesis.

Diffuse large noncleaved cell lymphoma

In this study, four cases of diffuse, large noncleaved cell lymphoma have been analyzed. This includes cases 2, 3, 6, and 9. All 4 cases exhibited multiple chromosomal abnormalities.

Cytogenetic analysis of case 2 which is a case of diffuse, large noncleaved cell lymphoma, intermediate grade showed 3 consistent abnormalities involving

chromosomes 1, 14 and 18. The presence of 1p+, 14q+ and 18q- was quite apparent. It is not possible to say exactly whether the extra chromosomal material on chromosome 14 is from chromosome 18, once again due to the poor quality of the G-banded chromosomes.

Southern blot analysis indicates that there is a *bcl-2* gene rearrangement. Therefore it is highly probable that the 14q+ is a t(14;18) chromosomal translocation. Three bands are detected in this lane. The rearrangement may also occur at the major breakpoint region on the *bcl-2* gene.

Case 3 is another diffuse lymphoma, but it is of an intermediate to high grade type. Cytogenetic analysis indicates that there is an increase in the number of chromosome abnormalities observed than there is in the previous case which is an intermediate grade lymphoma. Along with the 14q+ marker chromosome, the presence of a trisomy 11 with a del(11)(q13q14), del(13)(q14), and del(15)(q25→qter) is also observed. The deletion sites are significant since at these sites, specific proto-oncogenes and a tumor suppressor gene are located. The *bcl-1* proto-oncogene is located on chromosome 11q13. The tumor suppressor gene is located on the 13q14 and the *fes* gene is located on the region of chromosome 15q25. It appears that more than one proto-oncogene or transforming gene are involved in the progress of this disease from an intermediate to a high grade.

In the Southern analysis, 2 bands are detected; one of which is approximately 5.7 kb. It represents the germline band. Another band which is observed at the 2 kb region possibly represents the rearranged *bcl-2* gene. From the Southern and the cytogenetic analysis it is not possible to determine where the *bcl-2* gene has been

moved. Cytogenetic analysis does not show the 14q+ marker chromsome to a t(14;18). The extra chromosomal material on chromosome 14 remains unknown. But, *bcl-2* gene rearrangements were detected through the Southern analysis.

Case 6 and 9 are also diffuse large noncleaved cell lymphomas of intermediate grades. Both cases exhibit multiple chromosomal abnormalities. In case 6 it appears that the disease may be beginning to approach a high grade type. A few cells have been observed to contain del(13)(q14), and the 14q+ marker chromosome. This is similar to case 3.

The *bcl-2* gene does not appear to be involved since the Southern blot does not show any *bcl-2* rearrangements. Only one band is observed and this is the germline band.

Case 9 also exhibits various nonconsistent chromosomal abnormalities. Whether the *bcl-2* gene is involved could not be determined since there was no DNA sample available.

Follicular small cleaved cell lymphoma

Three cases of follicular small cleaved cell lymphoma have been analyzed cytogenetically. These include cases 4, 8, and 10. All three cases exhibited the t(14;18)(q32;q21) chromosomal translocation.

In case 4, trisomy 7, t(14;18(q32;q21) and an unique t(8;11)(p21;q13) chromosome abnormalities are observed. This case is in transition from a low grade to an intermediate grade (Bal et al., 1990). The presence of trisomy 7, as observed in case 1, might also allow the cells a growth advantage. The unusual t(8;11)(p21;q13) chromosomal translocation may be indicative of tumor progression. It is possible that the *bcl-1*

proto-oncogene may be involved in same way since it is at the rearrangement site on chromosome 11q13.

Southern blot analysis of this case does show that *bcl-2* gene rearrangement does occur. But in this sample of DNA no germline band is visible on this blot. This is perhaps due to the fact that the population of cytogenetically normal lymphocytes are considerably lower than the abnormal cells.

Case also had the expected t(14;18) chromosomal translocation. Various other abnormalities were also detected. Since it is a low grade lymphoma, the number of chromsomal abnormalities are rather low.

Southern analysis shows *bcl-2* rearrangement as well as the germline band. The labelled *bcl-2* probe hybridizes to a 19.1 kb and a 8.2 kb band which are the rearranged bands. The 5.7 kb band represent the germline bands.

Finally, case 10 which is also a low grade follicular lymphoma, exhibits a t(14;18) translocation. Only in this case, it has very few consistent chromosomal abnormalities other than the t(14;18).

In situ hybridization with the tritiated *bcl-2* probe demonstrates positively that it is a t(14;18). There is a significant number of grains accumalated on the 14q+ marker chromosome. This implies that the *bcl-2* gene has moved from chromosome 18 to chromosome location 14q32.

Diffuse small lymphocytic lymphoma

Only one case of diffuse small lymphocytic lymphoma with plasmacytoid features was examined. This case, case 5, had various random chromosomal abnormalities.
Whether
any of the abnormalities observed is significant cannot

be determined due to the lack of metaphase cells.

No *bcl-2* gene rearrangement was detected in the Southern blot analysis. Only a single band was observed. This band represents the germline, unrearranged gene.

Follicular large cleaved cell lymphoma

There was only one case of follicular large cleaved cell lymphoma, case 7, of intermediate grade. The expected t(14;18) was not observed in tbe cytogenetic analysis. This supports studies that show that the *bcl-2* gene rearrangement must occur early in B-cell maturation. It is probably through some other mechanism through which this case has progressed. There were various chromosomal abnormalities observed but the significance of this cannot be determined since the number of cells in metaphase was quite low.

Southern analysis only shows the germline band. Therefore, in this case no *bcl-2* gene rearrangement has occured.

From the Southern analysis, cases 1, 2, 3, 4, and 8 exhibited the *bcl-2* gene rearrangement. In case 1, 2, and 8, three major bands were detcted. It is most likely that the rearrangment has occurred within the major breakpoint region of the *bcl-2* gene. To determine positively which of the rearranged fragments is the fragment containing the *bcl-2*--IgH gene, this particular blot would have to be rehybridized with a specific IgH probe. Since it is known that in most cases, the *bcl-2* major breakpoint rearanges with the $J_H$region, the probe would have to contain the J_H sequence.

Case 3 which shows 2 bands, one being the germline band. The breakpoint has occurred on either side of the 3.5 kb *bcl-2* genomic fragment used as the probe. If the

breakage had occurred within the fragment , then a total of 3 bands would be detected. One band would represent represent the germline and the other two would represent the rearranged fragments.

DNA from peripheral blood lymphocytes (PBL) from unaffected individual was also probed with this *bcl-2* gene fragment. It only showed hybridization to occur to the germline fragment. No other signal was detected, indicating no *bcl-2* rearrangement.

With the combination of cytogenetic and molecular analysis it is possible to study the progression of the disease. From this study it can be seen that as the disease progresses from a low grade to a high grade, there is an increase in the number of chromosomal abnormalities detected in the cytogenetic analysis. This supports other studies where an increase in chromosomal abnormalities have been observed (Cabanillas et al., 1989; Schouten et al., 1990; Richardson et al., 1987). All of the six out of ten cases the presence of the 14q+ marker chromosome was prevelant. Only case 3 did not have a t(14;18), translocation, but *bcl-2* rearrangement was detected in the Southern analysis. It suggests that some diffuse lymphomas might arise from a follicular center cell stage. A recent study does show that *bcl-2* does not have to rearrange within the IgH locus but it can also recombine with the immunoglobulin κ chain gene (Osada et al., 1989).

The detection of t(14;18) in most of these cases supports studies that imply that this abnormality occurs early in the process which leads the cell to become neoplastic. As more and more abnormalities accumulate, the disease becomes more of the agressive type.

Since *bcl-2* gene recombination is observed in both

follicular and diffuse lymphomas it seem to indicate that the *bcl-2* gene has a significant role in the cell cycle. This rearraangement of *bcl-2* gene to the IgH locus causes an increase in its message which in turn causes an increase in the product. During B-cell development, an increase in *bcl-2* transcript is detected at the early stages of development. As the B-cell matures into an Ig producing plasma cell, the *bcl-2* transcript levels drop. As the level drop, the Ig transcripts increase. This is exzpected since the mature B-cell's main function is to produce immunoglobulins. Therefore when the *bcl-2* gene is brought to the IgH locus, the levels of *bcl-2* transcript is maintained as the B-cell matures. This is one of many steps which will then cause the cell to continue to proliferate instead of maturing into the next stage of development.

Though other proto-oncogenes have not been used in this study, cytogenetic analysis has indicated the possibility of other transforming genes to be involved as well as a tumor suppressor gene. Besides the *bcl-2* activation, other oncogenes can be activated in similar fashion and thereby clinically and histologically transform malignant lymphomas from a low grade to a high grade (Lee et al., 1989; Vaux et al.,1988).

VII. SUMMARY

The cytogenetics and molecular aspects of nonHodgkin's lymphoma was studied. From this study various chromosomal abnormalities were detected using the standard G-banding technique. The involvement of chromosome 14 and chromosome 18 in translocation to produce the t(14;18)(q32;q21) has a very significant role

in diseases of this type. The recombination of the *bcl-2* gene, located on chromosome 18q21, has a significant role in both follicular and diffuse lymphomas. This has been demostrated by both in situ hybridization technique and Southern blot analysis.

VIII. CONCLUSION

From this study, it has been observed that chromosomal abnormalities such as deletion, translocation and trisomies are the main abnormalities detected in the ten cases of nonHodgkin's lymphoma. However, it is not possible to positively correlate such findings with specific lymphoma types. A large number of case would have to be examined before a positve correlation can be made.

Through the use of in situ hybridization technique and Southern blot analysis movement of the *bcl-2* gene was studied. This study further supports other studies that indicate the importance of this gene in nonHodgkin's lymphoma. Transloction between chromosomes 14 and 18 has been determined to be the major chromsomal abnormality detected in NHL.

In conclusion, cytogenetic and molecular studies in lymphoma can become an important tool. With high resolution techniques,not only chromosomes but recurrent breakpoint sites will be better defined. The significance of chromosomal abnormalities can be related with oncogenes. Molecular biology with cytogenetic studies will contribute to the better understanding of the malignancy process.

IX. REFERENCES

Abe, R., Hayashi, Y., Sampi, K., Sakurai, M.: Burkitt's Lymphoma with 2/8 Translocation: A Case Report with Special Reference to the Clinical Features. Cancer Genet Cytogenet 6: 135-142, 1982.

Aisenberg, A. C., Wilkes, B. M., Jacobson, J. O.: The *bcl-2* Gene is Rearranged in Many Diffuse B-cell Lymphomas. Blood 71: 969-972, 1988.

Alitato, K., Schwab, M.: Oncogene Amplification in Tumor Cells. Adv Cancer Res 47: 235-281, 1986.

Armitage, J, O., Sanger, W.G., Weisenburger, D. D., Harrington, D. S., Linder, J., Bierman, P. J., Vose, J. M., Purtilo, D. T.: Correlation of Secondary Cytogenetic Abnormalities With Histologic Appearance in Non-Hodgkin's Lymphomas Bearing t(14;18)(q32;q21). J Natl Cancer Inst 80: 576-580, 1988.

Bal, S., Williams, G., Ray, M.: A Case of Follicular Small Cleaved Cell Lymphoma having a t(14;18) and t(8;11). Cancer Genet Cytogenet 1990 (In Press).

Berger, R. Bernheim, A., Weh, H. J. Flandrin, G., Daniel, M., Brouet, J. C., and Colbert, N.: A New Translocation in Burkitt's Tumor Cells. Hum Genet 53: 111-112, 1979.

Berger, R., Berhheim, A., Valensi, F., Flandrin, G.: 22q- and 8q- in a Non-Burkitt Lymphoma. Cancer Genet Cytogenet 8: 91-92, 1983.

Berger, R., Flandrin, G., Bernheim, A., Valensi, F., Sigaux, F., Daniel, M., Ochoa-Naguera, M. H.: Cytogenetic Studies of 32 Malignant Lymphomas. Non-Hodgkin's Lymphomas: New Techniques and Treatments. 4th Cancer Research Workshop, Grenoble, pp. 79-86, 1984.

Cabanillas, F., Pathak, S., Grant, G., Hagemeistser, F., McLaughlin, P., Swan, F., Rodriguez, M., Trujillo, J., Cork, A., Butler, J. J., Katz, R., Bourne, S., Freireich, E.: Refractoriness to Chemotherapy and Poor Survival Related to Abnormalities of Chromosome 17 and 7 in Lymphoma. American J Med 87: 167-172, 1989.

Capra, J.D. Tucker, P. W.: Human Immunoglobulin Heavy Chain Genes. J Biol Chem 264: 12745-12748, 1989.

Chen-Levy, Z, Nourse, J. & Clearly, M. L.: The *bcl-2* Candidate Proto-Oncogene Product Is a 24kDa Integral-Membrane Protein Highly Expressed in Lymphoid Cell Lines & Lymphoma Carrying t(14;18). Mol Cell Biol 9: 701-710, 1989.

Chenevix-Trench, G.: The Molecular Genetics of Human Non-Hodgkin's Lymphoma. Cancer Genet Cytogenet 27: 191-213, 1987.

Cory, S.: Activation of Cellular Oncogenes in Hemopoietic Cells by Chromosome Translocation. Adv Cancer Res 47: 189-234, 1986.

Croce, C. M., Shander, M., Martinis, J., Cicurel,L., D'Ancona, G. G., Dolby, T. W., Kaprowski, H.: Chromosomal Location of the Genes for Human Immunoglobulin Heavy Chains. Proc Natl Acad Sci (USA) 76: 3416-3419, 1979.

Dalla-Favera, R., Bregni, M., Erikson, J., Patterson, D., Gallo, R. C., Croce, C. M.: Human *c-myc* onc Gene is Located on the Region of Chromosome 8 that is Translocated in Burkitt's Lymphoma Cells. Proc Natl Acad Sci (USA) 79: 7824-7827, 1982.

Dalla-Favera, R.: Translocation and Rearrangement of the *c-myc* Oncogene in Human Undifferentiated B-cell Lymphomas. Science 219: 963-967, 1983.

Dani, C. Blanchard, J. M., Piechaczyk, M., El Sabouty, S., Marty, L., Jeunteur, P.: Extreme Instability of *myc* mRNA in Normal and Transformed Human Cells. Proc Natl Acad Sci (USA) 81: 7046-7050, 1984.

Donti, E., Brunangelo, F., Bordoni, S., Rosetti, A., Carloni, I., Donti, G. V.: ins(6;11) In a Case of Peripheral T- Cell Lymphoma. Cancer Genet Cytogenet 27: 367-369, 1987.

Erikson, J., Martinis, J., Croce, C. M.: Assignment of the Genes for Human Lambda Immunoglobulin Chains to Chromosome 22. Nature 294: 173-175, 1981.

Fleishman, E.W., Prigogina, E. L., Ilynskaya, G. W., Probatova, N. A., Konstantinova, L. N., Kruglova, G. V., Volkova, M. A., Omanov, D. S.: Chromosomal Characteristics of Malignant Lymphoma. Hum Genet 82: 343-348, 1989.

Fraisse, J., Vasselon, J. C., Petit, J. C., Brizard C. P.: Cytogenetics in Non-Hodgkin's Malignant Lymphomas.---Sotto, Vrousos, Sotto, Vincent (eds.), Non-Hodgkin's Lymphomas: New Techniques and Treatments, 4th Cancer Res. Workshop, Grenoble 1984, pp. 73-78 (Karger, Basel 1985)

Gaunt, K. L., Callaghan, J., Roberts, D. F.: Karyotype Abnormalities in Non-Hodgkin's Lymphomas. Ann Genet 29: 82-87, 1986.

Graninger, W. B., Seto, M., Boutain, B., Goldman, P., Korsmeyer, S. J.: Expression of Bcl-2 and Bcl-2--Ig Fusion Transcripts in Normal and Neoplastic Cells. J Clin Invest 80: 1512-1515, 1987.

Griesser, H., Thachuk,D., Reis, M. D. Mak, T. W.: Gene Rearrangements and Translocations in Lymphoproliferative Diseases. Blood 73: 1402-1415, 1989.

Halder, S., Beatty, C., Tsujimoto, Y., Croce, C. M.: The *bcl-2* Gene Encodes a Novel G Protein. Nature 342: 195-198, 1989.

Hamuster, H., Adolf, S.: In Situ Hybridization Studies On Variant t(2;8) Translocations In Burkitt's Lymphoma Lines. Hum Genet 73: 73-76, 1986.

Kaneko, Y., Abe, R., Sampi, K., Sakurai, M.: An Analysis of Chromosome Findings in Non-Hodgkin's Lymphomas. Cancer Genet Cytogenet 5: 107-121, 1982.

Kaprowski, H., Herlyn, M., Balaban, G., Parmiter, A., Ross, A., Nowell, P.: Expression of the Receptor for Epidermal Growth Factor Correlates with Increased Dosage of Chromosome 7 in Malignant Melanoma. Somatic Cell Mol Genet 11: 297-302, 1985.

LeBeau, M. M., Rowley, J. D.: Recurring Chromosomal Abnormalities in Leukemia and Lymphoma. Cancer Surveys 3: 371-394, 1984.

Lee, M., Blick, M. B., Pathak, S., Trujillo, J. M., Butler, J. J., Katz, R., McLaughlin, P., Hagemeister, F. B., Velasquez, W. S., Goodacre, A., Cork, A., Gutterman, J. U., Cabanillas, F. The Gene Located at Chromosome 18 Band q21 is Rearranged in Uncultured Diffuse Lymphomas as Well as Follicular Lymphomas. Blood. 70: 90-95, 1987.

Lee, J. T., Innes, D. J., Williams, M. E.: Sequential *bcl-2* and *c-myc* Oncogene Rearrangements Associated with the Clinical Transformation of NonHodgkin's Lymphoma. J Clin Invest 84: 1454-1459, 1989.

Lenoir, G. M., Preud'homme, J. L., Berheim, A., Berger, R.: Correlation Between Immunoglobulin Light Chain Expression and Variant Translocation in Burkitt's Lymphoma. Nature 298: 474-476, 1982.

Leroux, D., Jalbert, H., Bar, J., Monteil, M.: Translation (4;11) in a Case of Malignant Lymphoma. Cancer Genet Cytogenet 36: 159-163, 1988.

Levine, E., Arthur, D. C., Frizzera, G., Peterson, B. A., Hurd, D. D., Bloomfield, C. D.: There Are Differences in Cytogenetic Abnormalities Among Histologic Subtypes of the Non-Hodgkin's Lymphomas. Blood 66: 1414-1422, 1985.

Levine, E. G., Arthur, D. C., Machnicki, J., Frizzera, G., Hurd, D., Peterson, B., Gajl-Peczalska, K. J., Bloomfield, C. D.: Four New Recurring Translocations in Non-Hodgkin Lymphoma. Blood 74: 1796-1800, 1989.

Lukes R.: The Pathological Picture of the Malignant Lymphomas, in Proceeding of the International Conferance on Leukemia-Lymphoma. C. J. D. Zarafonetis, ed. pp. 333-356, 1968. Lea and Febiger, Philadelphia.

Malcolm, S., Barton, P., Murphy, C., Ferguson-Smith, M. A., Bentley, D. L., Rabbitts, T. H.: Localization of Human Immunoglobulin Light Chain Variable Region Genes to the Short Arm of Chromosome 2 by In Situ Hybridization. Proc Natl Acad Sci (USA) 79: 4957-4961, 1982.

Maniatis, T., Fritsch, E. F., Sambrook, J.: Molecular Cloning--A laboratory Manual. pp. 382-389, 1982. Cold Spring Harbor Laboratory.

Manolov, G., Manolova, Y.: Marker Band in One Chromosome 14 from Burkitt's Lymphomas. Nature. 237: 33-34, 1972.

Manolova, Y., Manolov, G., Kieler, J., Levan, A., Klein, G.: Genesis of the 14q+ marker in Burkitt's Lymphoma. Hereditas 90: 5-10, 1979.

Mark, J., Ekedahl, C., Dahlenfors, R.: Characteristics of the Banding Patterns in Non-Hodgkin and Non-Burkitt Lymphomas. Hereditas 88: 229-242, 1978.

McBride, O. W., Heiter, P. A., Hollis, G. F., Swan, D., Otey, M., Leder P.: Chromosomal Location of Human Kappa and Lambda Immunoglobulin Light Chain Constant Regions. J Experimental Med 155: 1480-1490, 1982.

McDonnell, T. J., Deane, N., Platt, F. M., Nunez, G., Jaeger, U., McKearn, J. P., Korsmeyer, S. J. *bcl-2*--Immunoglobulin Transgenic Mice Demonstrate Extended B-cell Survival and Follicular Lymphoproliferation. Cell 57: 79-88, 1989.

McKeithan, T. W.: Molecular Biology of Non-Hodgkin's Lymphomas. Seminars in Oncology 17: 30-42, 1990.

Mead, G. M.: Clinical Aspects of Early Stage of Non-Hodgkin's Lymphoma. Brit J Cancer 61: 7-8, 1990.

Melchers, F. Potter, M.: Mechanisms of B-cell Neoplasia 1987--Workshop at the Basel Institute for Immunology pp. 7-315, 1987. Editionnes <Roche>, Basle, Switzerland.

Morton, C. C., Taub, R., Diamond, A., Lane, M. A., Cooper, G. M., Leder, P.: Mapping of the Human *Blym-1* Transforming Gene Activated in Burkitt Lymphomas to Chromosome 1. Science 223: 173-175, 1984.

Naylor, S. L., McGill, J. R., Zabel, B. V.: In Situ Hybridization of Metaphase and Prometaphase Chromosomes. Methods in Enzymology 151: 279-292 1987.

Nowell, P. C.: What Causes Lymphocytic Tumors? Cancer Invest 8: 49-57, 1990.

Nowell, P. C., Hungerford, D. A.: Chromosome Studies on Normal and Leukemic Human Leukocytes. J Natl Cancer Inst 25: 85-93, 1960.

Ohyashiki, K., Yoshida, M. A., Ohyashiki, J., Kock, F., Han, T., Sandberg, A. A: Two 14q+ Chromosomes in Malignant Lymphoma: Crucial Cytogenetic Changes on 14q. Cancer Genet Cytogenet 17: 325-31, 1985.

Osada, H., Seto, M.,Ueda, R., Emi, N., Takagi, N., Obata, Y., Suchi, T., Takahashi,T.: *bcl-2* Gene Rearrangement Analysis in Japanese B-cell Lymphomas; Novel *bcl-2* Recombination with Immunoglobulin α Chain Gene. Jpn J Cancer Res 80: 711-715, 1989.

Panami, A., Ferti-Passantonopoulou, A., Deronvenoulas, J.: Partial Duplication of 1q in a Malignant Lymphoma. Cancer Genet Cytogenet 11: 87-90, 1984.

Reed, J. C., Tsujimoto, Y., Alpers, J. D., Croce, C. M., Nowell, P. C.: Regulation of bcl-2 Proto-Oncogene Expression During Normal Human Lymphocyte Proliferation. Science 236: 1295-1299, 1987.

Reed, J. C., Tsujimoto,Y., Epstein, S. F., Cuddy, M., Slabiak, T., Nowel, C. P., Croce, C. M.,: Regulation of *bcl-2* Gene Expression in Lymphoid Cell Lines Containing Chromosomes. Oncogene Res. 4: 271-281, 1989.

Reeves, B. R., Pickup, V. L.: The Chromosome Changes in Non-Burkitt Lymphomas. Hum Genet 53: 349-355, 1989.

Richardson, M. E., Quanguang, C., Filippa, D. A., Offit, K., Hampton, A., Koduru, P., Jhanwar, S., Lieberman, P. H., Chaganti, R. S. K.: Intermediate to High Grade Histology of Lymphomas Carrying t(14;18) is Associated with Additional Nonrandom Chromosome Changes. Blood 70: 444-447, 1987.

Robbins, S. L., Cotran, R., Kumar, V.: Pathologic Basis of Disease. pp. 653-704, 1984. Third Ed. Saunders, Philadelpia.

Rowley, J. D.: The Philadelphia Chromosome Translocation --A Paradigm for Understanding Leukemia. Cancer 65: 2178-2184, 1190.

Saltman, D. L., Cachia, P. G., Dewar, A. E., Ross, F. M., Krajewski, A. S., Ludlum, C., Steel, C.M.: Characterization of a New Non-Hodgkin's Lymphoma Cell Line (NCEB-1) With a Chromosomal (11;14) Translocation [t(11;14)(q13;q32)]. Blood 72: 2026-2030, 1988.

Sandberg, A.A.: Chromosome Changes in the Lymphomas. Hum Pathol 12: 531-539, 1981.

Schouten, H. C., Sanger, W. G., Weisenburger, D. D., Anderson, J., Armitage, J. O.: Chromosomal Abnormalities in Untreated Patients with Non-Hodgkin's Lymphoma; Association with Histology, Clinical Characteristics and Treatment Outcome. Blood 75: 1841-1847, 1990.

Seto, M., Jaeger, U., Hockett, R. D., Graninger, W., Bennett, S., Goldman, P., Korsemeyer, S. K.: Alternative Promoters and Exons, Somatic Mutations and Deregulation of *bcl-2*--Ig Fusion Gene in Lymphoma. EMBO J 7: 123-131, 1988.

Speaks, S. L., Sanger, W. G., Linder, J., Johnson, D. R., Armitage, J. O., Weisenburger, D., Purtilo, D.: Chromosomal Abnormalities in Indolent Lymphoma. Cancer Genet Cytogenet 27:335-344, 1987.

Taub, R., Kirsch, I., Morton, C., Lenoir, G., Swan, D., Tronick, S., Aaranson, S., Leder, P.: Translocation of the *c-myc* Gene into the Immunoglobulin Heavy Chain Locus in Human Burkitt's Lymphoma and Murine Plasmacytoma Cells. Proc Natl Acad Sci (USA) 79: 7837-7841, 1982.

Tilly, H., Bastard, C., Halkin, E., Lenormand, B., Biget, M., Dauce, J., Lees, O., Monconduit, M., Piquet,H.: Del(14)(q22) in Diffuse B-cell Lymphocytic Lymphoma. Am J Clin Pathol 89: 109-113, 1988.

Tsujimoto, Y., Finger, L. R., Yunis, J., Nowell, P. C., Croce, C. M.: Cloning of Chromosome Breakpoints of Neoplastic B-cells with the t(14;18) Chromosomal Translocation. Science 226: 1097-1099,1984.

Tsujimoto, Y., Jaffe, E., Cossman, J., Gorham, J., Nowell, P. C., Croce, C. M.: Clustering of Breakpoints on Chromosome 11 in Human B-cell Neoplasms with the t(11;14) Chromosome Translocation. Nature 315: 340-343, 1985a.

Tsujimoto, Y., Gorham, J., Cossman, J., Jaffe, E., Croce, C. M.: The t(14;18) Chromosome Translocations Involved in B-cell Neoplasm Result from Mistakes in VDJ Joining. Science 229: 1390-1393, 1985.

Van Den Berghe, H., Parloir, C., Gosseye, S., Englebienne, V., Cornu, G., Sokal, G.: Variant Translocation in Burkitt's Lymphoma. Cancer Genet. Cytogenet 1: 9-14, 1979.

Vaux, D. L., Cory, S., Adams, J. M.: Bcl-2 Gene promotes haemopoietic cell survival and cooperates with c-myc to immortalize pre-B cells. Nature 335: 440-442, 1988.

Working Formulation: National Cancer Institute Sponsored Study of Classification of NonHodgkin's Lymphoma. Cancer 49: 2112-2135, 1982.

Yunis, J. J.: Symposium on Chromosomes and Neoplasia. Hum Pathol 12: 494-516, 1981.

Yunis, J. J., Oken, M. M., Kaplan, M. E., Ensrud, K. M., Howe, R. R., Theologides, A.: Distinctive Chromosomal Abnormalities in Histologic Subtypes of Non-Hodgkin's Lymphoma. N Engl J Med 307: 1231-1236, 1982.

Yunis, J. J., Frizzerra, G., Oken, M. M., McKenna, J., Theologides, A., Arnesen, M.: Multiple Recurrent Genomic Defects in Follicular Lymphoma: A Possible Model for Cancer. N Engl J Med 316: 79-84, 1987.

Yunis, J. J., Mayer, M. G., Arnesen, M. A., Aeppli, D. P., Oken, M. M., Frizzera, G.: Bcl-2 and Other Genomic Alterations in the Prognosis of Large Cell Lymphoma. N Engl J Med 320: 1047-1054, 1989.